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The Chromosome Constitution of Human Marrow in Various Developmental and Blood Disorders

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IMPROVED SPREADING TECHNIQUES for mammalian chromosomes have enlivened cytogenetic investigation of karyotype anomalies in developmental defects and nuclear variability in neoplasia. Recent controversy about the correct human somatic chromosome number (Tjio and Levan, 1956; Ford and Hamerton, 1956; Kodani, 1957; Stern, 1959a) now appears settled overwhelmingly in favor of 46 (Chu, 1960), although more meiotic data on Japanese individuals are needed to rule out somatic elimination of Kodani's supernumeraries (Makino and Sasaki, 1959). The diploid human idiogram has been elaborated in surprising detail; it permits identification of the X and Y chromosomes with a fair degree of certainty (Chu and Giles, 1959), and has served as the norm for the unravelling of apparent inconsistencies between the interphase sex-chromatin and phenotype (Barr, 1959 a and b).

The heredity of certain developmental anomalies which had defied decades of speculative debate was—entirely during the course of 1959—clarified in chromosomal terms, thanks to teamwork between physicians, geneticists and cytologists in a few English, French and Swedish laboratories. An undercurrent of international competition has accelerated the tempo of publications in this important enterprise. At any rate, England's "present lead in human chromosome studies" (Anonymous editorial, Lancet, 1959) seems a more civilized subject for national pride than championship in anti-human ballistics.

The priorities in the human chromosome field are recorded in an excellent recent discussion by Nachtsheim (1959). There is, however, need for a more inclusive summary, such as the present compilation of our findings in 42 human marrows with all available data on various developmental disorders and leukemias.

METHODS

Marrow cells were secured by aspiration of the sternal or iliac marrow. The puncture area was infiltrated with novocaine and 1 to 2 ml. of marrow was

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drawn into a sterile syringe. In the study of developmental anomalies (but not for the leukemic preparations) we employed the short-term culture method of Ford, Jacobs and Lajtha (1958), with the following modifications: Earle's solution was used instead of Krebs-Ringer, and the patient's own serum was added to the 5% glucose in isotonic saline, in place of AB serum. Marrow specimens were obtained during the afternoon; they were then held for about 9 hours at room-temperature in a waterbath in which a heating and recirculation device turned itself on at 1:30 a.m., quickly achieving a constant temperature of 37°C ±0.5°C. After 7 hours, sufficient colchicine was added to make a culture concentration of 0.005%, and incubation was continued for a further 1½ to 2 hours. No growth failures occurred in any of the marrows so processed. Incubation was followed by brief treatment with hypotonic citrate. Fifty per cent acetic acid gave better fixation than acetic alcohol. Acetic orcein was used for staining in preference to the more cumbersome Feulgen technique. Orcein staining was also found more suitable than the Schiff reaction for demonstration of X-chromosomal heteropycnosis.

After failing with the above method to obtain sufficient numbers of leukemic mitoses for chromosome study, the leukemic marrows were not incubated, but were aspirated into Earle's solution, immediately treated with hypotonic citrate, fixed and squashed without prior use of colchicine. Well-spread, countable metaphases, representative of the marrow population components existing *in vivo*, were usually obtainable by this direct technique.

Sex-chromatin and "drum-sticks" were examined in smears of buccal mucosa and blood, whenever necessary for diagnostic purposes.

RESULTS AND DISCUSSION

1. Normal marrows

Marrow cells from 10 normal subjects, 5 men and 5 women, had the diploid chromosome number of 46 in 90% of 307 exact metaphase counts. The chromosome counts in the diploid range appear on line 1 of Table 1. Figures 1 and 2 depict the normal female and male karyotypes.

All marrows contained up to 4% polyploid metaphases, ranging from tetraploid to 32-ploid. The polyploid cells were interpreted as dividing megakaryocytes or osteoclasts (Fig. 3). Somatic pairing of homologous chromosomes in diploid cells occurred with a frequency of about 0.3%. In these metaphases, the haploid number of bivalent chromosome pairs was established.

Figure 4 is from a normal female marrow and shows altogether 23 bivalents. It should be noted that the thickness of these laterally paired units is considerably greater than in diploid metaphases, such as Figures 9–14, which are reproduced at the same magnification. Twenty-four units can be distinguished in a similar cell (Fig. 5) from the marrow of a Klinefelter's patient (see below) with a chromosome constitution of 47 (XXY). Judged by their thickness, there are 21 autosomal bivalents. The 2 smallest autosomal homologues, situated near upper center and at about 7 o'clock, have failed to pair. The probable XXY

TABLE 1. BONE MARROW CHROMOSOME CONSTITUTIONS IN NORMAL SUBJECTS AND VARIOUS DEVELOPMENTAL DISORDERS

(based on 1458 exact counts in the diploid range)

Number of cases	Diagnosis	Total metaphase counts	% Frequency of chromosome numbers 43	44	45	46	47	48	49
10(5♀, 5♂)	Normal controls	307	—	3	5	90	2	—	—
7*	Turner's (XO)	444	0.5	5	86	8	0.5	—	—
1*	Turner's (XO and XX?)	34	6	2	50	42	—	—	—
1*	Turner's (XO or XX?)	110	—	1	49	45	4	1	—
1*	Turner's (XO)	30	—	—	10	70	13	7	—
4†	Klinefelter's (XXY)	86	—	—.	—	1	87	11	1
2*	Testicular deficiency (XY, XXY?)	100	—	1	4	57	38	—	—
1*	Intersex (XY)	85	—	5	13	82	—	—	—
1♀†	Precocious puberty	54	—	—	2	22	52	22	2
1♀†	Precocious puberty	27	—	—	8	88	4	—	—
2♂*	Mongolism	86	—	—	1	8	90	1	—
2♂*	Lowe's syndrome	29	—	—	3	97	—	—	—
1♀†	Pseudohypoparathyroidism	66	—	2	6	90	2	—	—

* = buccal mucosa negative for sex-chromatin
† = buccal mucosa positive for sex-chromatin

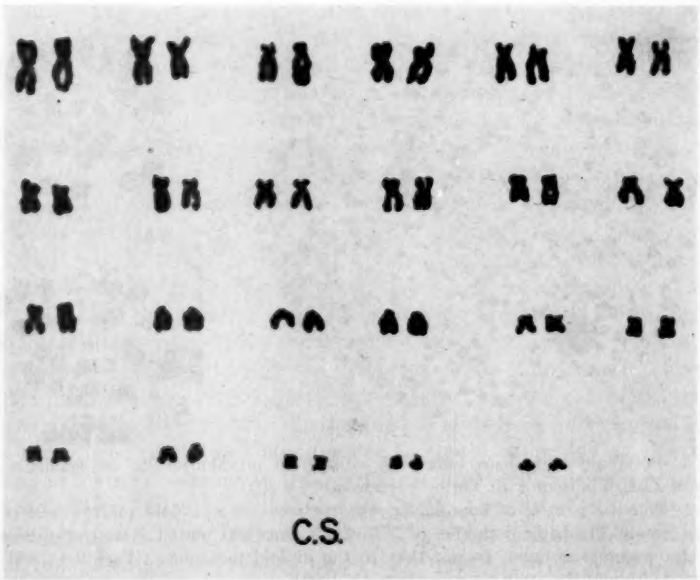


FIG. 1—Diploid idiogram (2n = 46) from marrow of C.S., a normal female. The first chromosome pair in the second row is probably the X pair. (× 2100).

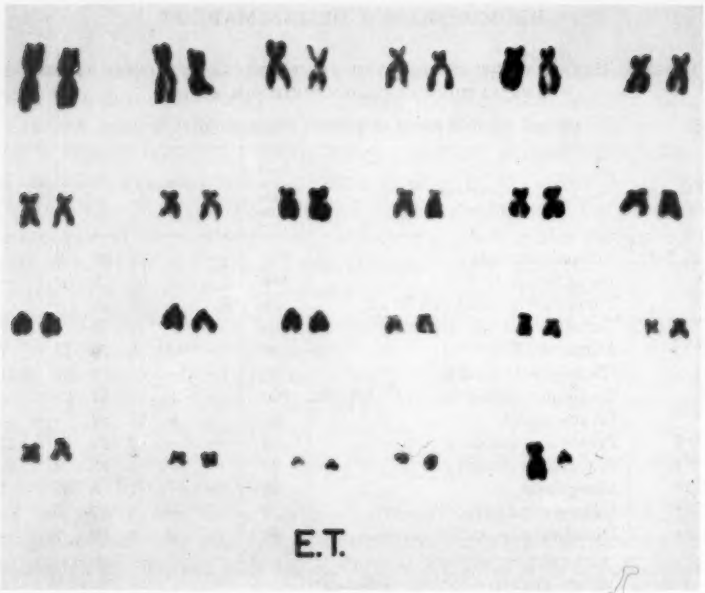


FIG. 2—Diploid idiogram (2n = 46) from marrow of E.T., a normal male. The fifth chromosome pair in the bottom row is interpreted as XY. (× 2200).

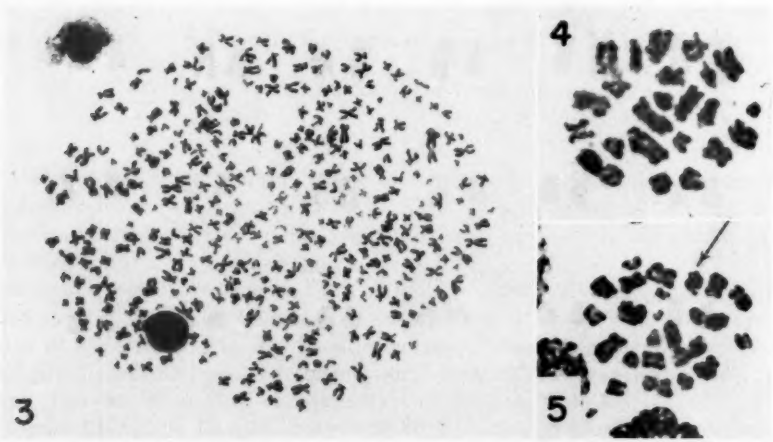


FIG. 3—Polyploid metaphase (16n − 8 = 360) of megakaryocyte or osteoclast from marrow of J.M , a patient with Turner's syndrome. (× 300).

FIG. 4—Somatic pairing of homologous chromosomes in a diploid marrow cell of C.S., a normal female. The haploid number of 23 bivalents may be counted. Note that thickness of paired chromosomes is much greater than in the diploid metaphases Figs. 9–14 which are reproduced at about the same magnification. (× 1200).

FIG. 5—Somatic pairing of homologues in a diploid marrow cell of N.W., a patient with Klinefelter's syndrome. This metaphase plate is interpreted as being composed of 24 units: 21 bivalent autosome pairs, 2 small unpaired autosomes and the XXY trio (arrow). (× 1200).

trio—2 laterally paired X-chromosomes and the small Y, terminally attached to the left-hand X as in male meiosis—is marked by an arrow. Figures such as these are rare and their interpretation is, of course, debatable. However, they have been observed and analyzed in detail often enough to rule out the possibility that they are accidental approximations of the haploid number resulting from asymmetric mitosis or broken metaphase plates.

Similar "haploid" mitoses were described in rat myelocytes by Kinosita et al. (1954). Since no haploid prophases were found in our human marrows, somatic pairing in diploid nuclei is either an abortive phenomenon, or it may initiate somatic reduction to haploidy followed by nuclear degeneraton, such as occurs in normoblastic maturation.

2. Marrow karyotypes in abnormal developmental conditions

A. Ovarian Agenesis (Turner's Syndrome): Our criteria for diagnosing Turner's syndrome were threefold: no evidence of ovarian function, buccal mucosa negative for sex-chromatin, certain accessory anomalies (webbing of the neck, cubitus valgus, congenital heart disease, growth retardation). Since the latter conditions vary greatly from case to case, somatic mosaicism for the XX and XO condition was considered as a possible contributory factor to this variability. However, the most obvious explanation for the diverse clinical manifestations is phenotypic expression of a variety of recessive traits carried on the single X, 45 (XO).

Difficulties in the precise identification of the submetacentric human X-chromosome, which ranks about 5th to 7th in length, have been admitted by most cytologists. In Turner's and in Klinefelter's syndrome, and less distinctly in normal female tissues, this difficulty is to some extent overcome by the pronounced heteropycnosis and allocycly of the X, not previously seen in man. An allocyclic replication and contraction rate is to be inferred from the characteristic staining properties of the X during different mitotic stages in certain female tissues of mouse, rat, Chinese hamster and other mammals (Ohno et al., 1959; Ohno and Hauschka, 1960; Kato and Yerganian, 1959). For a more detailed discussion of this allocycly, recently demonstrated by differential uptake of tritium thymidine in the X-chromosomal DNA of the grasshopper (Lima de Faria, 1959) and the hamster (Taylor, quoted by Yerganian, 1959), the paper by Ohno and Hauschka (1960) should be consulted.

Two extremes of X-allocycly may be seen in the idiograms of Figures 6 and 8. Figure 6 shows the single heteropycnotic, tightly contracted and intensely stained X on the right of the third row. Figure 8 shows abnormal lagging in the replication of the last chromosome in row 6, identified as the X. The doubling of all the autosomes in this metaphase is clearly complete, while the X appears to have failed in replicating its DNA. Usually, X-allocycly is less extreme, as apparent from Figures 9–13. In late prophases and beginning metaphases, the most intensely stained unit often meets the criteria for arm-length and size described for the X by Chu and Giles (1959). Heteropycnosis, therefore, may serve as an additional useful (though not unequivocal) attribute in the identification of the sex-chromosomes.

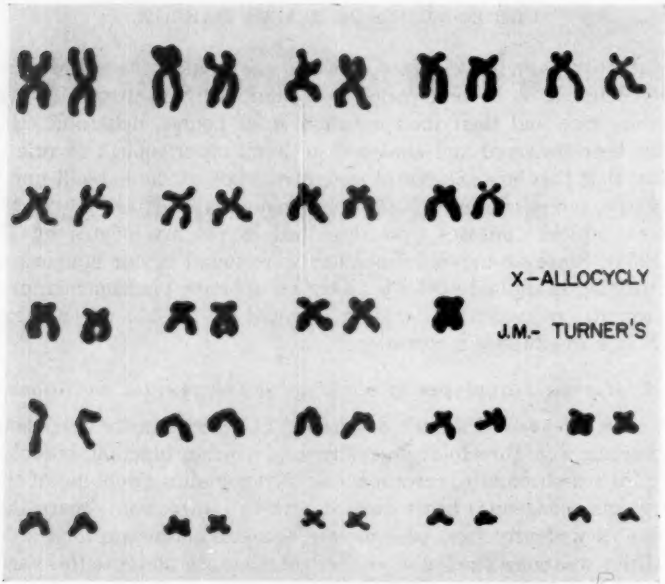


FIG. 6—Hypodiploid idiogram ($2n - 1 = 45$) from marrow of J.M., a patient with Turner's syndrome. The single, heteropycnotic X-chromosome lies at the right of the third row. This idiogram was prepared from the metaphase shown in Fig. 10. (× 2000).

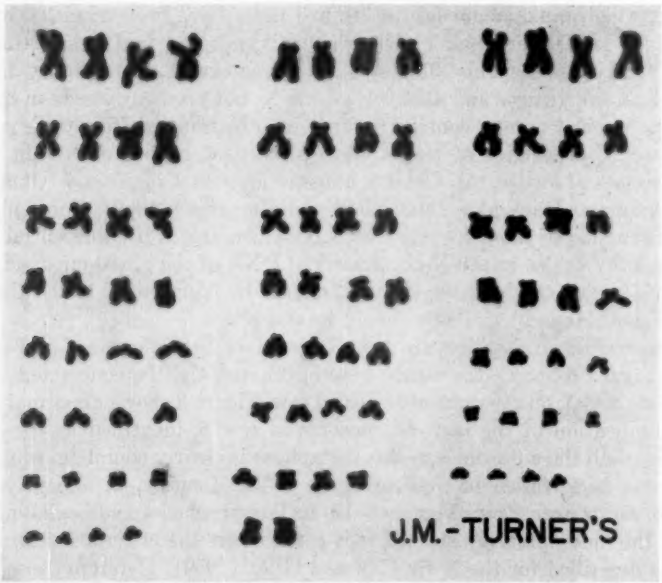


FIG. 7—Hypotetraploid idiogram ($4n - 2 = 90$) from marrow of J.M., a patient with Turner's syndrome. The two chromosomes at the right of the bottom row are believed to be the two X-chromosomes. (× 1400).

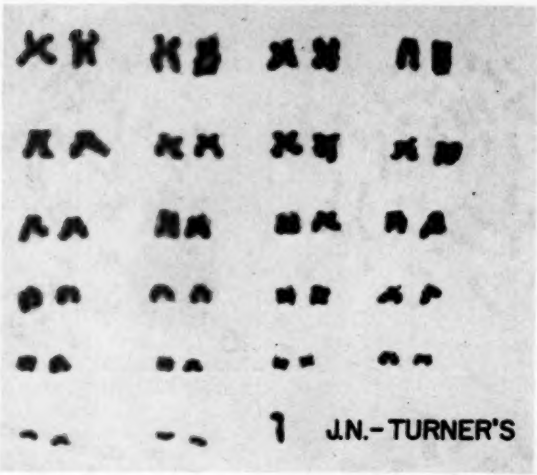


FIG. 8—Hypodiploid idiogram ($2n - 1 = 45$) from marrow of J.N., a patient with Turner's syndrome. Extreme allocycly in the replication rate of the single X-chromosome is evident from the as yet not clearly divided X at the right of the bottom row. As a rule, the X does not lag nearly as far behind the autosomes. (× 1600).

We have analyzed a representative series of 10 Turner's patients (Table 1, lines 2–5). In 7 of them, the modal chromosome constitution was 45 (XO), as first described by Ford et al. (1959b) and subsequently by others (Table 2, lines 1–5, Figs. 6, 7, 8).

In 2 of our subjects with the characteristic syndrome of ovarian agenesis, the chromosome constitution was about half 45 (XO) and half 46 (presumably XX). The 2 major karytoypes in the mosaic marrow on line 4 of Table 1 were imbalanced not merely with regard to the X-chromosome (either XO, or normal X plus incomplete X^1, as indicated by arrows in Fig. 11); they were either monosomic or trisomic for one of the smallest autosomes, as evident from the uneven number of small chromosomes in 10 metaphases suitable for detailed analysis.

On clinical grounds, the 38-year-old individual listed on line 5 of Table 1 was an unusual Turner's patient: 6 feet tall, muscular, weighing 214 pounds. While only 10% of her marrow cells contained 45 chromosomes, she was definitely of the XO constitution, the modal number of 46 resulting not from XX, but from trisomic representation of one of the two *largest* autosomes. This case will be studied further to determine whether her exceptional marrow idiogram extends to other somatic tissues.

During the "Second Symposium on Nuclear Sex" at King's College, London, Ford's discussion of 1 certain and 2 possible XX/XO mosaics with Turner's syndrome stimulated an "acute difference of opinion" (Anonymous editorial, Lancet, 1959). This skepticism concerning somatic mosaicism in man is no longer justified. Besides Ford's 3 cases, there are now on record 4 mosaic marrows

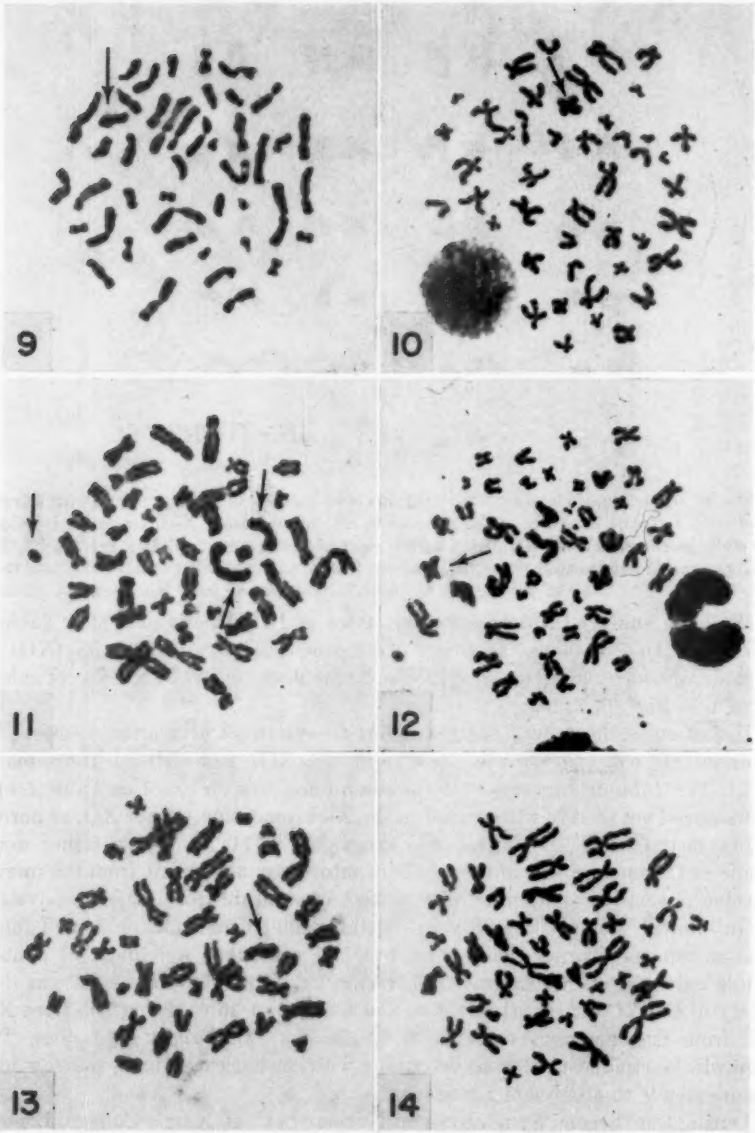


FIG. 9—Hypodiploid pro-metaphase (2n − 1 = 45) from marrow of J.M., a patient with Turner's syndrome. Arrow points to the heteropycnotic X-chromosome. (× 1500).

FIG. 10—Hypodiploid metaphase (2n − 1 = 45) from marrow of J.M., a patient with Turner's syndrome. Arrow points to the heteropycnotic X-chromosome. (× 1500).

TABLE 2. SUMMARY OF CHROMOSOME CONSTITUTION IN 19 CASES OF TURNER'S SYNDROME

Number of cases	Sex chromatin		Cytologic findings		Reference
	Buccal mucosa or skin	Blood	Characteristic chromosome number	Sex chromosomes	
1	—	n.t.*	45(98%)	XO	Ford et al., 1959b
1	—	n.t.	45(96%)	XO	Jacobs and Keay, 1959
2	—	n.t.	45	XO	Fraccaro et al., 1959
1	—	n.t.	45(100%)	XO	Tjio et al., 1959
3	—	n.t.	45	XO	Fraccaro et al., in press
1	—	n.t.	45 and 46	XO, XX(?)	" " " in press
7	—	+ or −	45(86%)	XO	Present report
2	—	+	45(50%), 46(43%)	XO and XX(?)	" "
1	—	++	45(10%), 46–48 (90%)	XO	" "

* = not tested

from patients with ovarian agenesis (Table 2, lines 6, 8, 9) and 3 mosaics in Klinefelter's syndrome (Table 3, lines 4, 7). Thus, a fairly high proportion of these abnormal individuals exhibits karyotypes that presuppose *somatic* nondisjunction during embryogenesis or during later life, possibly superimposed on sex-chromosomal abnormalities originating from *meiotic* nondisjunction.

While sex-linked marker genes may tell us which X-chromosome has been eliminated, they allow no absolute decision between elimination in a parental gonad or during ontogeny. Since the observed somatic mosaicism may well extend into tissues other than the marrow, it detracts somewhat from the reliability of sex-linked recessive color-blindness as evidence for X-chromosomal nondisjunction during maternal vs. paternal *meiosis*. That both may occur seems, nevertheless, clear from the hereditary analyses of Stern (1959b) and Stewart (1959).

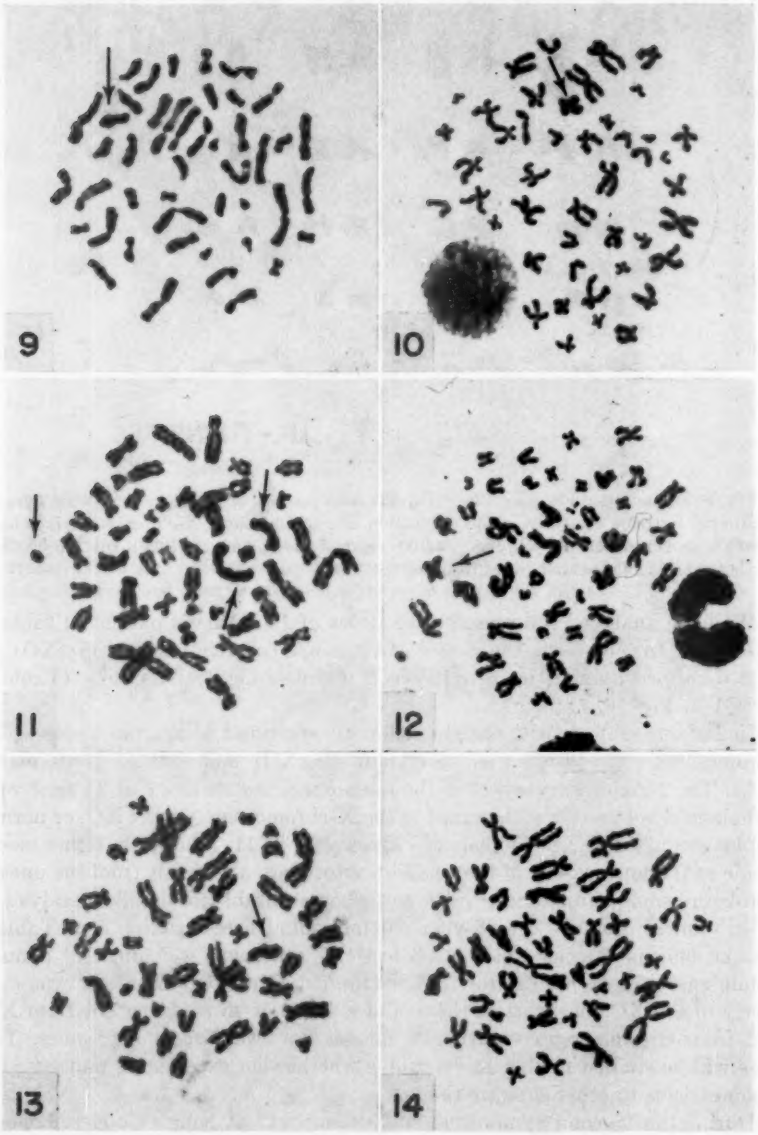
Our own series includes a colorblind Turner's individual whose brother and maternal grandfather had defective red-green color vision, but whose mother

FIG. 11—Hyperdiploid metaphase (2n − 1 + 2 = 47) from mosaic marrow of G.G., a patient with Turner's syndrome. The arrow at the left points to a small chromosome for which the cells of this patient are either tri- or mono-somic. Arrow at upper right indicates the heteropycnotic X-chromosome, a piece of which may have been translocated to the larger heteropycnotic unit below it, which is perhaps a second X. Another possible interpretation is that this metaphase is XO, in which case the stem-cell type with 47 chromosomes would be trisomic for one of the four largest chromosomes. (× 1500).

FIG. 12—Diploid metaphase (2n = 46) from marrow of N.W., a case with testicular deficiency. The shape and heteropycnosis of the unit indicated by arrow suggests that it is the X-chromosome. (× 1500).

FIG. 13—Diploid metaphase (2n = 46) from marrow of N.W., a case with testicular deficiency. The heteropycnotic X-chromosome is indicated by arrow. (× 1500).

FIG. 14—Hyperdiploid metaphase (2n + 1 = 47) from marrow of D.L.S., a patient with Klinefelter's syndrome. (× 1500).



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3	—	n.t.	45	XO	Fraccaro et al., in press
1	—	n.t.	45 and 46	XO, XX(?)	" " " in press
7	—	+ or −	45(86%)	XO	Present report
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1	—	++	45(10%), 46–48 (90%)	XO	" "

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from patients with ovarian agenesis (Table 2, lines 6, 8, 9) and 3 mosaics in Klinefelter's syndrome (Table 3, lines 4, 7). Thus, a fairly high proportion of these abnormal individuals exhibits karyotypes that presuppose *somatic* nondisjunction during embryogenesis or during later life, possibly superimposed on sex-chromosomal abnormalities originating from *meiotic* nondisjunction.

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Our own series includes a colorblind Turner's individual whose brother and maternal grandfather had defective red-green color vision, but whose mother

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FIG. 12—Diploid metaphase (2n = 46) from marrow of N.W., a case with testicular deficiency. The shape and heteropycnosis of the unit indicated by arrow suggests that it is the X-chromosome. (× 1500).

FIG. 13—Diploid metaphase (2n = 46) from marrow of N.W., a case with testicular deficiency. The heteropycnotic X-chromosome is indicated by arrow. (× 1500).

FIG. 14—Hyperdiploid metaphase (2n + 1 = 47) from marrow of D.L.S., a patient with Klinefelter's syndrome. (× 1500).

TABLE 3. SUMMARY OF CHROMOSOME CONSTITUTION IN 15 CASES OF KLINEFELTER'S SYNDROME, TESTICULAR DEFICIENCY, AND INTERSEX

Number of cases	Sex chromatin in buccal mucosa	Cytologic findings		Reference
		Characteristic chromosome number	Sex chromosomes	
1	+	47	XXY	Jacob's and Strong, 1959
4	−	46	XY	Jacobs et al., 1959c
1 (also a Mongol)	+	48	XXY	Ford et al., 1959a
1	+	46 and 47 (68%)	XXY and XX(?)	Ford et al., 1959c
1	−	46	XY	Ford et al., 1959c
4	+	47	XXY	Present report
2	−	46(57%) and 47	XY and XXY(?)	" "
1 (Intersex)	−	46	XY	" "

(a carrier) and father had normal vision. This patient's marrow contained more than 90% 45 (XO) cells. The single X-chromosome was obviously inherited from her mother. In this instance it may be claimed that fertilization of a normal ovum by an O-sperm, produced after nondisjunction or asynapsis of XY during paternal meiosis, was responsible for the XO constitution.

B. Testicular Dysgenesis (Klinefelter's Syndrome) and Testicular Deficiency: Only 4 patients with definite physiological and anatomical deficiencies of the testes, and at the same time showing positive sex-chromatin in their buccal mucosa, are here classified as Klinefelter's syndrome (Table 1, line 6). In the marrows of these subjects, the expected mode of 47 (XXY), as first established by Jacobs and Strong (1959), predominated (Fig. 14). A testicular biopsy from one of these individuals contained no trace of spermatozoa, spermatogonial mitoses or meiotic stages.

While 5 cases of "testicular feminization", examined by Jacobs et al. (1959c) and Ford et al. (1959c) were consistently 46 (XY) with negative buccal mucosa, our 2 subjects with testicular deficiency (not feminized) had mosaic marrows (Table 1, line 7). The modal cell type, shown in Figures 13 and 14, was 46 (XY); but more than 30% of the metaphases had 47 chromosomes and probably contained XXY. These two interesting cases resemble Klinefelter's syndrome clinically and suggest the possibility of somatic loss of one of the two X's.

Table 3 summarizes the pertinent data for 15 cases in this general category.

C. Precocious Puberty and Intersex: In one case of precocious puberty (Table 1, line 10), marrow had the normal chromosome constitution of 46 (XX). Another such patient examined by us had a record of menses and breast development since birth (Table 1, line 9). About half of her marrow metaphases had 47 chromosomes, the rest were evenly divided between 46 and 48. The modal cell type was trisomic for a chromosome in the general size-class of the X, and the patient may be tentatively considered as a triple-X female. By physiological and anatomical standards, this 9-year-old girl is better qualified for the designation "super female" than the masculinized patient shown by Jacobs et al. (1959b).

The marrow of an 18-year-old subject with intersex and negative buccal mucosa had a chromosome complement of 46 (XY). At laparotomy the following organs were identified: bilateral testes, bilateral fallopian tubes without a uterus, definite ovarian tissue on the right side and questionable ovarian tissue on the left. The external genitalia had intersexual aspects and there was no breast development. The XY karyotype of this individual, shown in Figure 15, differs from the only other intersex in the literature (Hungerford et al., 1959) where the chromosome constitution was undoubtedly 46 (XX).

D. Mongolism and Other Anomalies Not Involving Abnormal Sex-Chromosome Constitutions: Among the 22 Mongoloids so far studied cytologically in this and other laboratories, no departure from the trisomy of the smallest chromosome, discovered by LeJeune, Gauthier, and Turpin (1959a and b) has been found (Table 4). Figure 16 depicts the idiogram of a male Mongoloid examined by us.

Fifteen other human genetic and/or developmental defects fully or provisionally analyzed for somatic chromosome constitution include:

(a) One case of *polydysspondylism* with 45 chromosomes in which 2 autosomes had become attached to one another by translocation (Turpin et al., 1959).

(b) Fourteen anomalies in all of which the modal chromosome number was 46 and the gross chromosomal morphology appeared normal, viz.: one case each of *Marfan's syndrome, phenyl ketonuria, female pseudo-hermaphroditism, Gaucher's disease* (Tjio, Puck, and Robinson, 1959); four *anencephalic fetuses* (Harnden, Briggs, and Stewart, 1959); one case each of *epiloia, Laurence-Moon-Biedl syndrome, neurofibromatosis, arachnodactyly, osteogenesis imperfecta, achondroplasia* (Harnden, quoted in anonymous Lancet editorial, 1959), one *haemophilic* "girl" with male sex-chromatin pattern (Nilsson et al., 1959);

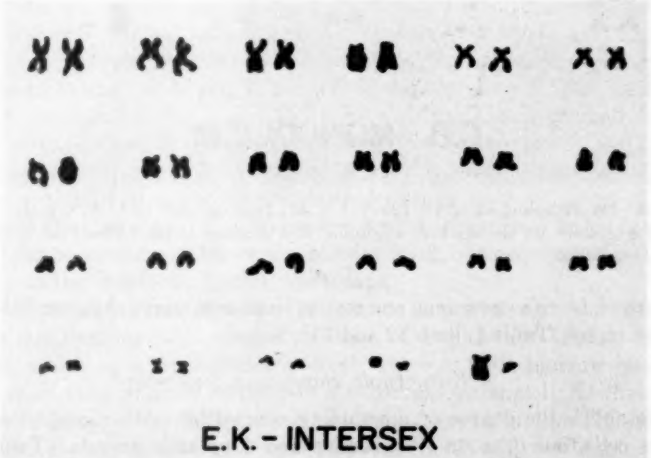

FIG. 15—Diploid idiogram (2n = 46) from marrow of E.K., an intersex. The fifth chromosome pair in the bottom row is interpreted as XY. (× 2000).

TABLE 4. SUMMARY OF CHROMOSOME CONSTITUTION IN 22 CASES OF MONGOLISM

Number of cases	Sex	Characteristic chromosome number	Idiogram details	Reference
10	6♂, 4♀	47	3 of smallest A*	Lejeune et al., 1959a,b
6	3♂, 3♀	47	3 of smallest A	Jacobs et al., 1959a
1	♂(Klinefelter's)	48	XXY + 3 smallest A	Ford et al., 1959a
3	2♂, 1♀	47	3 of smallest A	Böök et al., 1959
2	2♂	47	3 of smallest A	Present report

* A = Autosome

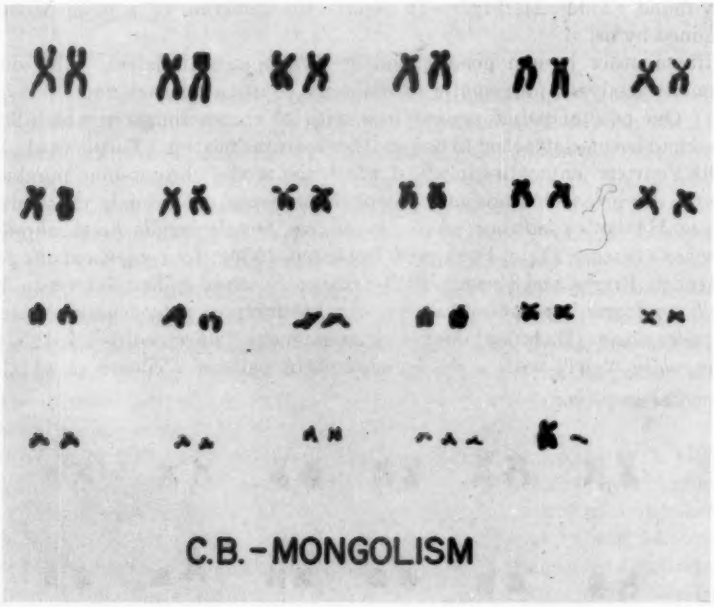

FIG. 16—Hyperdiploid idiogram (2n + 1 = 47) from marrow of C.B., a male mongoloid. The cell is trisomic for the smallest autosome, fourth group in the bottom row, to the left of the XY. (× 2000).

two cases of *Lowe's syndrome*, and one patient with *pseudohypoparathyroidism* (present report, Table 1, lines 12 and 13).

3. Karyotypic changes in leukemia

To establish the degree of aneuploidy compatible with viability of human marrow cells, our data on 7 leukemias and 1 aplastic anemia (Table 5) are here briefly discussed in relation to all other available findings in short-term tissue cultures of leukemic marrows and blood (Table 6).

After Nowell, Hungerford and Brooks (1958) had reported normal diploid chromosome constitutions in blood cultures from 3 cases with acute granulocytic leukemia, Ford, Jacobs and Lajtha (1958) reported observations of both numerical and structural chromosome anomalies in a "blast-cell leukemia". Baikie et al. (1959, 1960a, and b) examined a larger series. They found karyotypic changes in the stem-cell types of 4 among 5 acute leukemias, but normal chromosome conditions in 5 chronic myeloid leukemias, 1 chronic lymphatic leukemia, and 2 cases of myelomatosis. In considering the relationship of chromosomal anomalies to the pathogenesis of acute leukemia, they favored the view that "acute leukemias are the result of changes in the genetic material of the cell", whereas chronic leukemogenesis may involve "separate mechanisms of induction".

Ford and Mole (1959) questioned the latter assumption, pointing out the possibility that the normal metaphases seen in marrow-cultures from patients with chronic leukemia "were really normal marrow cells which must inevitably be found to some extent in aspirated bone marrow samples". This highly justified criticism applies, in our opinion, to neoplastic karyology by short-term tissue culture methods in general. Goldstein and Hauschka (unpublished) have counted chromosomes in human ovarian ascitic carcinoma and other tumors *before* (highly aneuploid) and *after* (mostly 46) brief tissue culture. We concluded that, even in relatively complete tissue culture media, the more exacting nutritional dependence of aneuploid neoplastic cells may enable normal host cells to overgrow them *in vitro*, or at least resume cellular multiplication before the unbalanced neoplastic karyotypes gain growth momentum.

For this reason, all our leukemic marrows were fixed immediately after procurement from the patient. The findings in Tables 5 and 6 (lines 8, 9) indicate a wider karyotypic variability and more frequent aneuploid modality among both chronic and acute leukemias than observed by other investigators.

A typical hyperdiploid idiogram from a chronic myelocytic leukemia (Table 5, line 7) is pictured in Figure 17. After matching 21 pairs, 6 chromosomes, probably including the X and Y, are left in the bottom row. Two of these are unusually small and 2 may be autosomes for which this set is trisomic.

The 5 chromosomes in the bottom row of Figure 18 represent a similar aneuploid excess over the tetraploid number of 92 in the modal idiogram of an entirely hypertetraploid, acute lymphoblastic leukemia.

Chromosomal imbalance of a degree that would probably be lethal in embryogenesis is thus not only viable on the cellular level, but may sometimes be contributory to the neoplastic growth advantage.

Since all the data summarized in Table 6 are no doubt valid, the discrepancy between our findings and, especially, those of Bayreuther (1960) raises the question: which cells are mitotically most active in fresh marrow biopsies, as against short-term marrow cultures in a relatively incomplete medium? Cytologic results obtained by both methods for each patient, and uncomplicated by transfusion, X-ray treatment or chemotherapy, should help settle this question.

Bayreuther (1960) completely discounts the carcinogenic potential of aneuploidy and considers the karyotypic irregularities observed in leukemias and

TABLE 5. CHROMOSOME CONSTITUTION OF LEUKEMIC, ANEMIC AND NORMAL HUMAN MARROWS
(based on 537 exact counts)

Case and diagnosis	Prior treatment	Marrow findings	Distribution of exact chromosome counts ← Diploid range → ← Tetraploid Range →																Total counts	% Cells with 46 chromosomes
			<44	44	45	46	47	48	49–53	54–91	92	93	94	95	96	97	98	99	>99	
D.M., 3½ yr. ♂ Acute lymphoblastic leukemia in complete remission	Steroids, dichloromethotrexate	Normal, normoblasts slightly increased	3	3	1	*46*	1	—	—	—	—	—	—	—	—	—	—	—	—	54	85
P.B., 12 yr. ♂ Acute lymphoblastic leukemia	Steroids, X-ray, transfusion	No megakaryocytes, over 95% lymphoblasts	—	—	—	—	—	—	—	—	—	—	1	—	3	*9*	4	4	2	23	0
R.D., 4 yr. ♂ Acute lymphoblastic leukemia	None	Few megakaryocytes, 75% lymphoblasts	—	1	3	8	*14*	3	2	—	—	—	—	—	—	—	—	—	—	31	26
R.A., 64 yr. ♀ Acute myeloblastic leukemia	Transfusion	Hypocellular, but many myeloblasts	—	2	*8*	4	—	—	—	—	—	—	—	—	—	—	—	—	—	14	29
A.L., 58 yr. ♂ Acute myeloblastic leukemia in relapse	Transfusion, steroids, amidazolethioguanidine	Rare megakaryocytes, few normoblasts, 76% myeloblasts	2	4	6	*36*	11	9	2	—	—	—	—	—	—	—	—	—	—	70	51
E.D., 71 yr. ♂ Chronic lymphocytic leukemia	None	Few megakaryocytes, >90% lymphocytes	—	—	3	*4*	—	—	—	*4*	—	—	—	—	—	—	—	—	—	11	36

J.McG., 57 yr. ♂ Chronic myelocytic leukemia in blastic phase	Transfusion, myleran colcemide	Increased myeloblasts and promyelocytes, few megakaryocytes and normoblasts	—	—	—	2	2	*12*	3	—	—	—	—	—	—	—	—	—	—	19	10
A.A., 24 yr. ♂ Aplastic anemia	None	Very hypocellular, mostly plasma cells, lymphocytes and histiocytes	—	—	—	1	*6*	1	—	—	—	—	—	—	—	—	—	—	—	8	12
10 Normal controls (5♂, 5♀)	None	Normal	—	8	18	*276*	5	—	—	—	(~4% polyploids, none counted)							—	—	307	90

TABLE 6. SUMMARY OF CHROMOSOME CONSTITUTION IN 35 HUMAN LEUKEMIAS

Number of cases	Diagnosis	Chromosome numbers determined in	Modal karyotype shows numerical and/or structural abnormalities	Reference
3	2 blast cell, 1 lymphatic	Marrow culture	1/3	Ford et al., 1958
5	Acute myelocytic	Marrow culture	4/5	Baikie et al., 1959 and
8	Chronic myeloid & lymphatic	" "	0/8	Baikie et al., 1960a,b
2	Acute myelocytic	Blood culture	0/2	Nowell & Hungerford,
2	Chronic myelocytic	" "	2/2	1960
5	Acute myeloid & lymphatic	Marrow culture	0/5	Bayreuther, 1960
3	Chronic myeloid & lymphatic	" "	0/3	" "
5	Acute myeloid & lymphatic	Marrow directly from patient	3/5	Present report
2	Chronic myeloid & lymphatic	Marrow directly from patient	2/2	" "

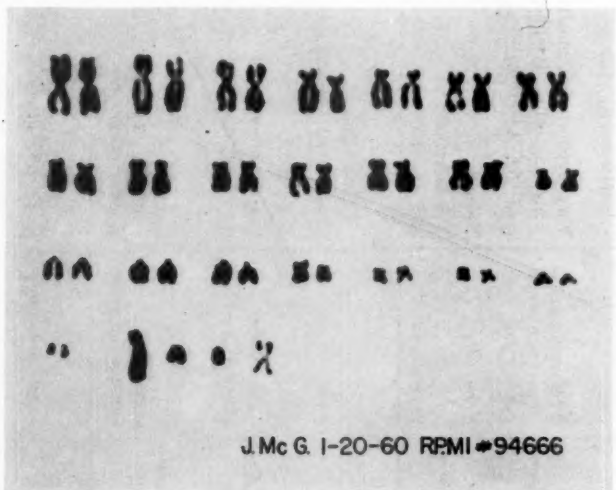

FIG. 17—Hyperdiploid leukemic idiogram (stem-cell type with 48 chromosomes) from marrow of J.McG., a male patient with chronic myelocytic leukemia in blastic phase. (× 1600).

other primary tumors as mere epiphenomena in the wake of a more fundamental cellular alteration. A reasonable alternative to this extreme viewpoint would be to accept chromosomal imbalance as one among several genetic and non-genetic mechanisms whereby a cell can achieve neoplastic autonomy. That aneuploid

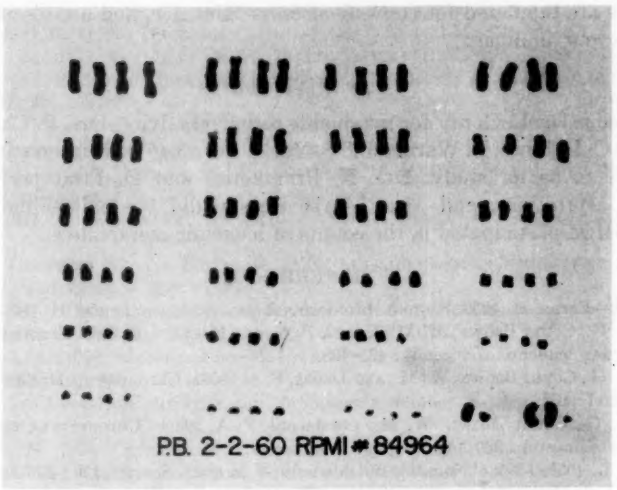

FIG. 18—Hypertetraploid leukemic idiogram (stem-cell type with 97 chromosomes) from marrow of P.B., a male patient with acute lymphoblastic leukemia. (× 1300).

cells and organisms may function within the precinct of relatively normal growth is, however, self-evident from the somatic karytoypes in some human developmental disorders.

SUMMARY

The chromosome pattern (number, morphology and frequency of chromosomal abnormalities) was examined in the marrows from 10 normal human subjects, 24 patients with developmental anomalies, 1 aplastic anemia, and 7 leukemias. In the 10 normal marrows, the chromosome number of 46 occurred with a frequency of 90% ± 1.7; about 0.3% true somatic pairing in diploids, and up to 4% polyploids were seen.

Essentially, our findings for ovarian agenesis, Klinefelter's syndrome and Mongolism agree with earlier published work. However, a larger-than-expected incidence of mosaicism, including karyotypes tri- or mono-somic for small as well as large autosomes, has come to light. Heteropycnosis and allocycly of the X-chromosome, not previously noticed in the human idiogram, are especially pronounced in Turner's syndrome. A probably triple-X, nine-year-old "superfemale" with precocious puberty, and a case of intersex with a male chromosome complement of 46 (XY) are described.

In 7 acute and chronic leukemic marrows, fixed immediately after sternal aspiration (rather than after temporary growth *in vitro*), aneuploidy was far more frequent than in normal controls. One leukemia was entirely hypertetraploid with a mode at 97, two had hyperdiploid modes at 47 and 48, and one had a hypodiploid cell population mode at 45 chromosomes.

All available chromosome data on human marrows in developmental and blood

disorders are tabulated for convenient cross-reference, and are discussed in relation to our findings.

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The Identification of Individual Chromosomes, Especially In Man*

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INTRODUCTION

WITH THE RECENT DISCOVERY that certain multiple anomalies in man are caused by specific abnormalities of the chromosome complement (for a brief review see Patau, Smith, Therman, Inhorn and Wagner, 1960) the reliable identification of individual chromosomes has become a matter of considerable genetical and perhaps medical interest. It might appear that the problem has already been solved. Tjio and Puck (1958) and, independently, Chu and Giles (1959) have published detailed accounts of the human chromosome complement, illustrated by exceptionally beautiful photographs, and concluded that in favorable cells every chromosome can be identified. These studies have enlarged our knowledge of the human chromosome complement but this particular claim can, regrettably, not be sustained. The fault lies not with the observations, which are above reproach, but with a method of quantitative evaluation that is as time honored among cytologists as it is misleading. The fact that the two publications support each other fairly well merely illustrates the point that repeated applications of a method with a built-in systematic error will tend to mislead in the same direction.

The method in question consists in the successive pairing-off of the chromosomes of a diploid nucleus by their measured length or by the lengths of their arms. It is then assumed that the pairs represent pairs of homologues. What has made this method so attractive to cytologists is no doubt its lack of ambiguity; two competent investigators who independently apply the method in the same manner to a given mitosis will usually arrive at the same result. This testifies to the accuracy of the length measurements but, unfortunately, not to the correctness of the interpretation of the obtained pairs as being pairs of homologues. It will be shown below that this interpretation is all too often a fallacy. By means of the pairing-off method idiograms of an almost endless number of plants and animals have been constructed and published. Understood as representations of the general morphology of chromosomes found in different karyotypes such diagrams are no doubt useful. However, their implication that all chromosomes of a set had been identified is more often than not unjustified.

In general, chromosomes have two arms separated by a centromere. In addition, there may be secondary constrictions and/or satellites and if these can be

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seen regularly, chromosome identification will be easy and certain. Usually, however, this must rest upon the lengths of the two arms unless it is preferred to replace these lengths by two other values: the total chromosome length and the arm ratio or arm index, i.e. the length of the long arm divided by that of the short one. This conversion has recently become the custom but it is difficult to see how it could aid identification. Conversion or no conversion, for quantitative purposes each chromosome yields but a pair of numbers, the equivalent of a point in a two-dimensional co-ordinate system. A graph in which every chromosome of a complement is represented by a point contains, therefore, all relevant information. It will be seen below that the plotting of such a graph is a remarkably effective method of demonstrating the limitations of the available information as well as of extricating all the information that may be accumulated by measuring the chromosomes of a sufficient number of cells.

The methodological points just touched upon will be elaborated in the first part of the present study. In the second part the graphical method mentioned above will play an essential role in an attempt to clarify to what extent individual chromosomes of man can be identified by current cytological techniques.

I. THE EVALUATION OF LENGTH MEASUREMENTS OF CHROMOSOMES

Sources of Variation in Length. Measurements can be made on drawings of individually centered chromosomes or on photomicrographs, the highest possible accuracy of both methods probably being about the same. Preferably each chromatid should be measured separately along its axis, and if there is an achromatic gap at the centromere it should be excluded. Carefully done measurements on technically suitable chromosomes can yield more accurate results than might be thought possible in view of the limited resolving power of the microscope. Because of this limitation there will be some uncertainty in the location, within the diffraction fringe, of the proximal and the distal end of each chromosome arm. However, if these locations are estimated with sufficient consistency, the systematic error caused by the optical uncertainty should be fairly constant and, therefore, for practical purposes negligible when chromosomes or arms of similar and not too short length are compared with each other. Of course, measurements of arms that are only a small fraction of a micron in length are next to meaningless. One can hardly take seriously the claim that one of the small acrocentric chromosomes in man has an arm ratio of 6.83 ± 0.17 ("95% level of fiducial significance") when it is realized that the total length of this chromosome at metaphase is usually less than 1.5μ which, with that ratio, would imply a length of the short arm of less than 0.19μ. Small wonder that other authors found quite different values to wit 3.67 and 2 0, for the arm ratio of the same chromosome. However, the lengths of most chromosome arms in man are large enough to justify the confidence that the measured lengths represent fairly well the actual lengths of these arms in the preparation.

The total length of all chromosomes, in other words, their average degree of contraction varies from cell to cell. Rothfels and Siminovitch (1958) have pointed out that a change in contraction need not affect smaller and larger chromosomes

in strict proportion to their length. In human bone marrow, especially after colchicine treatment, such "systematic differential contraction" can be readily observed. In cells with elongated chromosomes the medium-sized ones have about the same diameter as the largest chromosomes, but the latter are much thicker than the former in cells with highly contracted chromosomes. In metaphases with the lowest degree of chromosome contraction the chromosome length is probably more or less proportional to the length of the uncoiled chromonema and, therefore, to the approximate number of genes in the chromosome if this is an autosome, presuming, of course, that autosomes contain relatively little heterochromatin. The very short arms of acrocentric chromosomes are probably always thinner than the long arms. In addition, there is the possibility that these short arms, or major parts of them, belong to the heterochromatic region which is often found around the centromere. Therefore, the ratio of arm lengths, if it could be ascertained, would undoubtedly give an exaggerated impression of the gene content of these short arms. Their genetic role may be almost negligible.

When measurements on chromosomes from different cells are to be compared the variation in total chromosome length between cells becomes distracting. However, by expressing the length of each chromosome, or chromosome arm, in percent of the nuclear total we can eliminate this variation completely or at least to the extent that there is no disturbance from systematic differential contraction. Of this there can hardly be much, as measurements for identification purposes will usually be done on chromosomes which are not very contracted. It is possible, and indeed often advisable, to get rid of virtually all effects of systematic differential contraction by converting the length of each chromosome of a certain group into percent of the group total rather than of the nuclear total.

In the complement of man, there are a number of distinct groups within which the chromosomes are fairly similar. In suitable cells, it is easy to establish for every chromosome to which of these groups it belongs without any measurements. Serious problems of identification arise only within groups. If measurements are to help in solving such problems, the obtained lengths are best expressed as percent of the group total, for this will not only eliminate the irrelevant inter-group variation but will also make possible the use of cells in which the chromosomes of the particular group but not all of the other chromosomes are suitable for measurement. It shall henceforth be understood that "length" means relative length stated as percent of the nuclear total or of a group total, whichever the case may be. A conversion into percent, though not quite in the above form, has already been used by Rothfels and Siminovitch (1958) and by others following them.

If the above discussed sources of variation were the only ones affecting chromosome length, the measured lengths of two homologous and not too small chromosomes of the same cell ought to be almost identical. This they rarely are. Compare, for instance, the two chromosomes No. 2 in Fig. 5. Their lengths quite evidently differ, but it is also evident that the shorter one has chromatids that are somewhat thicker than those of the other chromosome. The length difference,

therefore, does obviously not imply a difference in chromosome mass. Such a variation in length, but apparently not in mass, that goes clearly beyond the variation due to inaccuracies of measurement is not unusual—as indeed one might expect. It is hardly conceivable that chromosomes in vivo, being moved around as they are, should not show some variation in the degree of contraction. It is even less conceivable that they should not suffer some distortion during preparation which includes their being spread in one plane. At any rate, whatever its source, there always is a length variation that no technical refinement can eliminate. The following example appears to be fairly typical.

Rothfels and Siminovitch (1958) have published the percent lengths of the two homologous chromosomes I in ten cells of the rhesus monkey. The identification of these chromosomes was certain, they are not only the longest of the complement but are also distinguished by a secondary constriction. Chromosomes more excellently prepared and more suitable for accurate measurements can hardly be imagined—yet their coefficient of variation proves to be 5.8 percent (computed from the differences of the two lengths per cell which yield an estimate of variance with 10 d.f.).

It so happens that the same coefficient of variation, 5.8%, is also found in the length measurements by Levan and Hsu (1959, Table 2) on ten pairs of chromosome No. 1 in man. This chromosome, too, can be identified with certainty and is, because of its length (which ranges in this case from 5.5 to 7.3μ), highly suitable for accurate measurements. It appears likely that 5.8 per cent is close to the irreducible minimum of chromosome length variation.

The Pairing-off Method

Because of the unavoidable length variation, measurements can merely yield estimates of the mean percent length of a given chromosome in comparably fixed cells. This mean, a population mean in the statistical sense, shall henceforth be called the "true" length of the chromosome. Homologous chromosomes have the same true length. Assume now a pair, P_1, of homologous chromosomes which exceed in true length the members of another pair, P_2, by an amount which is small relative to the standard deviation of length. Obviously, the shorter member of P_1 will then often be smaller than the longer member of P_2; in fact, if the four chromosomes are arranged according to their measured length any of the possible six different sequences of the members of the two pairs may result. If the pairs are very similar in true length, the two chromosomes with the largest measured length will not be homologous in almost two out of three cases. The pairing-off method, used as it commonly has been used, is likely to put together what does not belong together. It is also likely to create "statistically significant" differences where there are none, as the following fictitious, though essentially realistic, example will show.

Assume there were six chromosomes of identical true length per cell, their coefficient of variation being 5.8 percent (the value actually found in measurements on the largest chromosome of the rhesus monkey as well as of man—see above). Assume further a normal distribution of the chromosome length. Any

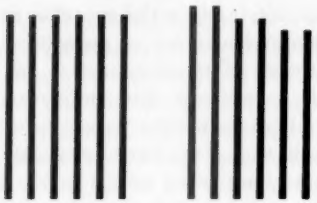

FIG. 1. Model. The pairing-off method, applied to five nuclei each with six chromosomes of identical true length (left) and a coefficient of variation of 5.8%, produces three pairs with significantly different lengths (right)—see text.

random sample of size six from a population of this kind may then be interpreted as the lengths of six chromosomes as they might have been found in a cell. By means of a normal distribution and table of random numbers five samples of six chromosomes each were obtained. To these data the pairing-off method was applied by calling the two longest chromosomes in each sample or cell "chromosome A," the next two "chromosome B," and the two smallest ones "chromosome C." The mean lengths of A, B, and C in the five "cells" are shown in Fig. 1, right. This diagram duplicates quite well the situation seen in many idiograms which had been constructed by means of the pairing-off method. It appears fair to conclude that in these cases the "demonstrated" differences between pairs were often as unreal as the ones in the present model.

Nothing could be more futile than to compute fiducial limits for the mean lengths of chromosomes "identified" by the pairing-off method in cases where the very existence of a difference in true length between any two successive pairs remains a conjecture. Yet this has been done recently for human chromosomes. How meaningless the thus obtained statistical backing is for the claim of having identified all human chromosomes can be seen from the fact that in the above model the differences between the chromosomes A, B and C are also statistically significant (A, B: $t_{18} = 3.79$; B, C: $t_{18} = 3.24$). This can readily be generalized. Any group of 2n chromosomes of identical true length will inevitably appear to consist of n pairs any two of which differ significantly from each other in length, if measurements of these chromosomes in a sufficient number of cells are subjected to the pairing-off method. The latter can do still more, it can always "identify" an odd chromosome such as the X chromosome in the male complement. If the pairing-off proceeds from the smallest to the largest chromosomes of the group with an odd number, the largest one will necessarily seem to be the odd chromosome. By the inverse process the smallest chromosome can be demonstrated to be the odd one.

The pairing-off method is potentially so misleading that its use cannot be justified except in the trivial case of pairs which differ so consistently from each other in all technically suitable, but otherwise unselected, cells that the homology of the members of each pair is self-evident. Of course, in such a case measurements are hardly necessary. This is not meant to depreciate the usefulness of measurements for the morphological analysis of difficult chromosome groups.

It will be shown below that there are methods of extracting reliable information from measurements on chromosomes of such groups but the pairing-off procedure is not one of them.

Thus far chromosomes have been treated as if they were characterized only by their total length. However, all that has been said remains essentially true when two quantities, such as the two arm lengths, are taken into account simultaneously.

The Karyogram

It has been pointed out in the introduction that a chromosome characterized by two quantities is best represented by a point in a two-dimensional co-ordinate system. The choice of these quantities—total length and arm index or two arm lengths, is in principle irrelevant but it is simpler to use the latter. Let the percent length, l, of the longer arm serve as abscissa and the percent length, s, of the shorter arm as ordinate. The scatter diagram obtained by plotting in this manner all chromosomes of the given complement will henceforth be referred to as a "karyogram." If points are entered only for the chromosomes of a special group, lengths now being given in percent of the group total, a "partial karyogram" will result.

A karyogram, although based on arm lengths, also shows the total chromosome

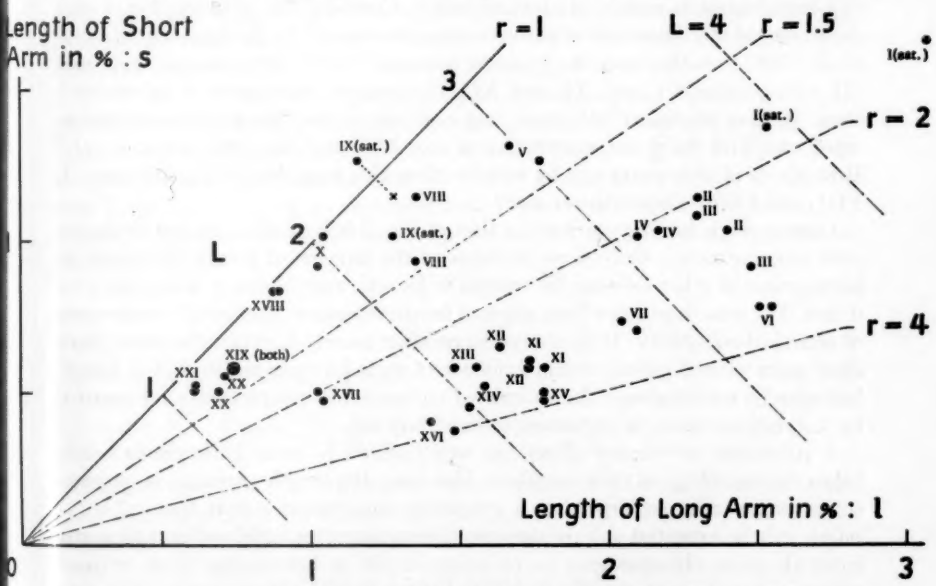

FIG. 2. Karyogram of rhesus monkey. All lengths in percent of total length of chromosome complement as measured by Rothfels and Siminovitch (1958, Table 1). Numbers of chromosomes as assigned by these authors. ×: location of indicated chromosome IX if its one arm had not been marked by satellite (see p. 256).

length, L = l + s, and the arm ratio r = l/s (Fig. 2). Experience so far indicates that two homologous chromosomes yield points in haphazard positions relative to each other. This means that points representing homologous chromosomes in the same cell can be regarded as a random sample of a bivariate distribution in which there is little, if any, correlation between l and s. The advantage of a karyogram is that it shows at a glance whether there are pairs of points clearly separated from all other points. If not, any attempt to identify chromosomes by length will be futile, at least in the given cell. If there are certain well defined pairs, the possibility should still be considered that mere chance has brought non-homologous chromosomes together. Of course, there may be one or more pairs within which homology is certain because of the presence of the same secondary constriction in both chromosomes. The distances between points which definitely or probably represent homologous chromosomes give some idea of the variation likely to exist between other homologues of similar size. In general, it is evident that the standard deviation of arm length increases with this length, though probably not proportionally. This relation, though undesirable for statistical purposes, need cause no concern as a detailed analysis is usually restricted to groups of fairly similar chromosomes.

To see in a karyogram the magnitude of the variation between homologous chromosomes can be a sobering experience, destroying at once any hope to identify homologues in certain clusters of points. Consider, for instance, Fig. 2 and the inscribed identifications of chromosomes as proposed by Rothfels and Siminovitch (1958) on the basis of the same numerical data. After excepting II and III ("contentious") and XI and XII ("proposed association is arbitrary") these authors continue: "All remaining pairings in this nucleus are straightforward, and with the possible exception of pair XIX and XX, even unequivocal." How much of this claim can be upheld when the variation within the pairs I, VIII, and IX is taken into account?

From a single karyogram such as that of Fig. 2 few conclusions can be drawn with any assurance. Only those features of the pattern of points that recur in karyograms of other cells can be trusted to be real, and that only under one condition. The cells must have been selected for measurement exclusively on grounds of technical suitability. If we choose to consider merely karyograms which show clear pairs we will indeed obtain evidence of sorts for their reality but it will be biologically meaningless. The sample of chromosome complements represented by karyograms must be unbiassed to be of any use.

A consistent recurrence of certain well isolated pairs in karyograms establishes the homology of their members. However, the length variation of chromosomes is generally so large that a consistent isolation of a pair from all other points can be expected only in the case of chromosomes which differ sufficiently from all other chromosomes to be recognizable as homologues even without measurement. Indeed, there is reason to believe that the identification of homologous chromosomes in individual cells can gain little, if anything, from length measurements. Especially in the case of small chromosomes the experienced cytologist may at times even draw valid distinctions where these do not show

up with sufficient clarity in measurements. If of two chromosomes of equal length, one appears more contracted and has an evidently higher Feulgen dye content than the other, it is justified to conclude that the two chromosomes do not have the same true length and are not homologous.

A Statistical Analysis of Measurements. It will be seen in the second part of this study that in man, as in most organisms, relatively few pairs of homologous chromosomes can at present be identified in individual cells. However, the characteristics of a chromosome which in individual cells can not be distinguished from other chromosomes of a certain group may nevertheless be analyzed statistically if individuals occur that have different numbers of this chromosome. For instance, a normal female has two X chromosomes, a male has one X; a trisomic has three chromosomes of a certain kind as compared with two in a normal person. Let the group containing the particular chromosome have g chromosomes in one type of individual and $g + 1$ in the other. The former type shall henceforth be called 'male' and the second 'female' but it should be understood that the subsequent considerations remain valid if we are dealing with normal and trisomic persons rather than with males and females. Whatever the special situation, it ought to be possible to deduce the size and the arm ratio of the extra chromosome in the 'female' by a statistical evaluation of length measurements on the chromosomes of the group in a sufficient number of 'male' and 'female' cells. How such an analysis may be approached will be discussed in the following. In principle these methods are extremely powerful, but they do require very extensive measurements if any degree of accuracy is to be achieved.

Let l' and s' be the true absolute lengths of the long and the short arm, respectively, of any given chromosome and let l'_x and s'_x be those of the extra chromosome, e.g. the X chromosome, in the 'female'. Let $S = \sum l' + \sum s'$ be the total length of all g chromosomes of the 'male' group. Then, $S + l'_x + s'_x$ is the total length of the $(g + 1)$ chromosomes of the 'female' group. Expressing all lengths in terms of the respective group total we obtain for the chromosomes of the 'male' group:

$$\left.\begin{aligned} l(m) &= \frac{l'}{S}; \qquad s(m) = \frac{s'}{S} \\ \text{and for those of the 'female' group:} \\ l(f) &= \frac{l'}{S + l'_x + s'_x}; \qquad s(f) = \frac{s'}{S + l'_x + s'_x} \end{aligned}\right\} \tag{1}$$

Consider now any two homologous chromosomes of which one belongs to the 'male' and the other to the 'female' group. The eqs. (1) yield relative arm lengths of the former which exceed those of the latter by a constant factor q:

$$l(m) = q\,l(f); \quad s(m) = q\,s(f) \tag{2}$$

$$q = 1 + l_x(m) + s_x(m) \tag{3}$$

Let $l_t(m) = \sum l(m)$ be the sum of the relative lengths of the long arms of

all g chromosomes of the 'male' group and $l_t(f) = \sum l(f)$ the corresponding sum, with $(g + 1)$ terms, of the 'female' group. Because of the left equation (2), $ql_t(f)$ contains every term of $l_t(m)$ and, in addition, $l_x(m)$. Hence:

$$l_x(m) = ql_t(f) - l_t(m) \tag{4}$$

So far, we have been considering true lengths. Assume now the chromosomes of the group have been measured in a number, N_m, of 'male' cells and in a number, N_f, of 'female' cells. For each cell the sum of the observed relative lengths of the long arms of all chromosomes of the group is determined. The means, $\overline{l_t(m)}$ and $\overline{l_t(f)}$, of these sums are then unbiassed estimates of the true values $l_t(m)$ and $l_t(f)$, respectively. Hence, for any given value of q the following represents an unbiassed estimate of the true value of the length of the long arm of the extra chromosome relative to the 'male' group total:

$$l_x(m) = q\overline{l_t(f)} - \overline{l_t(m)} \tag{5}$$

The statistics $ql_t(f)$ and $l_t(m)$ cannot be assumed to have identical variances, but if the number of chromosomes per group is as large as it is in the group containing the X chromosome in man ($g = 15$) the difference between the two variances will be negligible for practical purposes. Therefore, we may compute, for any value q, fiducial limits of $l_x(m)$ by means of the t distribution, using as standard error of $l_x(m)$:

$$s\,(l_x(m)) = \sqrt{\frac{q^2\,SS(f) + SS(m)}{N_f + N_m - 2}\left(\frac{1}{N_f} + \frac{1}{N_m}\right)} \tag{6}$$

wherein $SS(f)$ and $SS(m)$ represent the sum of squares of the N_f observed values $l_t(f)$ and that of the N_m observed values $l_t(m)$, respectively. From any pair of values q and $l_x(m)$ an estimate, $s_x(m)$, of the true value of the length of the short arm of the extra chromosome relative to the 'male' group total can immediately be obtained by means of eq. (3).

If nothing is known about q, the above computation can be performed for several values of q to yield points $(l_x(m), s_x(m))$ in the karyogram of the 'male'. These points determine a line which represents the estimated location of the extra chromosome. The fiducial limits define a strip in the karyogram. The extra chromosome can then be presumed to lie within that area in which this strip overlaps the area covered by the chromosomes of the group.

To estimate the value of q, and thereby the length of the extra chromosome, we may resort to a partial karyogram in which the co-ordinates are not the relative lengths of the long and of the short arm of each chromosome, but the logarithms of these lengths. Let $x = \log l$ and $y = \log s$. There will be a certain area, F, in the xy plane so that the likelihood of a chromosome of the 'female' group being found outside of F is smaller than a certain very small value. In the same manner an area M can be defined for the 'male' group. If the true location of a given chromosome in F is (x,y), that of any homologous chromosome in M must be, because of (2), $(x + c, y + c)$ wherein $c = \log q$. Thus, corresponding points in F and M can be transferred into each other by a shift along

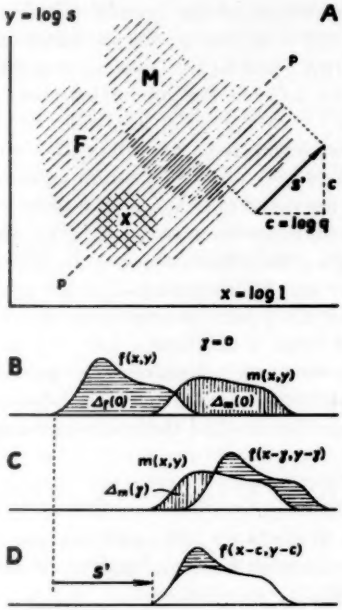

FIG. 3. A: distributions of "male" (M) and of "female" (F) chromosome group, the latter containing an extra chromosome (X). B–D: cross sections through distributions along line PP in A. See text.

a direction of 45° (Fig. 3, A). The problem of estimating q has now been reduced to that of estimating the size of this shift.

The simplest approach to this problem would be to plot in one logarithmic karyogram all $(g + 1)N_f$ chromosomes measured of the 'female' group and in another all gN_m chromosomes measured of the 'male' group and to determine the shift required to produce the maximal overlap of the two areas containing either set of points. This method is not recommended for two reasons. Firstly, it is inefficient as it makes no use of the information contained in the distribution of the points in the interior of these areas; secondly, it is not a generally unbiassed method, as a marginal location of the extra chromosome could cause F to be larger than M with the result that maximal overlap of those areas is likely to be achieved by a too small or by a too large shift, whatever the case may be.

Any efficient method for estimating the shift must take into account the distribution of all chromosomes of the group. For each chromosome there is a bivariate frequency distribution. When the g or $(g + 1)$ distributions are superimposed a distribution of expected numbers results. Let this distribution of the group in the case of the 'male' be given by $m(x,y)$ and in that of the 'female' by $f(x,y)$ (Fig. 3, B). The volume between the surface $m(x,y)$ and the xy plane is $\int m(xy)\, dx\, dy = g$, that between $f(x,y)$ and the xy plane is $\int f(xy)\, dx\, dy =$

$g + 1$. Consider now a shift, γ, of the 'female' distribution so that the shifted distribution $f_\gamma(x,y) = f(x - \gamma, y - \gamma)$ for all points (x,y). Let now $\Delta_m(\gamma)$ be the volume between $m(x,y)$ and $f_\gamma(x,y)$ over all areas in the xy plane in which $m(x,y) \geq f_\gamma(x,y)$ and let $\Delta_f(\gamma)$ be the volume between $f_\gamma(x,y)$ and $m(x,y)$ over all areas in which $f_\gamma(x,y) \geq m(x,y)$ (Fig. 3, C): $\Delta_m(\gamma) = \int(m(x,y) - f_\gamma(x,y))$ dx dy for $m(x,y) \geq f_\gamma(x,y)$ and $\Delta_f(\gamma) = \int (f_\gamma(x,y) - m(x,y))$ dx dy for $f_\gamma(x,y) \geq m(x,y)$. Evidently $\Delta_f(\gamma) - \Delta_m(\gamma) = 1$ for all values of γ. $\Delta_m(\gamma)$ assumes its minimum for $\gamma = c = \log q$ and it is $\Delta_m(c) = 0$ (Fig. 3, D).

If two observed distributions have somehow been derived from logarithmic karyograms, one with gN_m, the other with $(g + 1)\ N_f$ points, a statistic, $D_m(\gamma)$, corresponding to $\Delta_m(\gamma)$ can be computed. Because of the sampling variation, $D_m(\gamma)$ will as a rule not disappear for any value of γ but it can be minimized and thus an estimate of c can be obtained.

To transform the two scatter diagrams of points into distributions suitable for forming $D_m(\gamma)$ for a number of different γ values, identical square grids are laid upon the logarithmic karyograms and the points per square are counted.

Then

$$D_m(\gamma) = \sum (a(m) - (N_m/N_f)a_\gamma(f)) \qquad (7)$$

for all squares in which $a(m) > (N_m/N_f)a_\gamma(f)$ wherein $a(m)$ and $a_\gamma(f)$ are the numbers in identical squares of the 'male' and, after the shift γ, of the 'female' karyogram, respectively.

In the above procedure the choice of the grid poses a dilemma unless N_m and N_f are very large. If the grid unit is small, most squares may remain empty or contain just one point in which case $D_m(\gamma)$ may prove almost independent of the shift γ. If the grid unit is large, the minimizing of $D_m(\gamma)$ may yield different results depending on the, necessarily arbitrary, position of the grid.

The dilemma can largely be resolved by the following method. The grid unit is made relatively small, e.g. 0.025, but instead of entering for each square only the number of points lying within the square we add to this count the number of points in the eight surrounding squares. To determine, by means of (7), $D_m(\gamma)$ for different γ values it is simplest to proceed by increments of γ that are equal to the grid unit. In a set of actual measurements the following values were obtained: $D_m(0) = 253.0$; $D_m(0.025) = 184.5$; $D_m(0.05) = 222.0$. The parabola $D_m(\gamma) = 84\,800\,\gamma^2 - 4\,860\,\gamma + 253$ fits these three pairs of values. It assumes its minimum at $\gamma = c = 0.02866$. Hence $q = 1.068$.

It is realized that this method of estimating q is relatively crude, but it is believed that it is not appreciably biassed. It yields no information on the sampling error of q. To estimate this error it will be necessary to collect sufficient data for several independent determinations of q, preferably each to be based on the same number of cells, half of which should be 'male', the rest 'female'.

The statistical analysis of an extra chromosome that cannot be distinguished directly from other chromosomes of a certain group is, of course, not the only use to which chromosome measurements may be put legitimately. Obviously, *translocations* or other structural changes are apt to pose problems which can be

solved, if at all, only by a statistical evaluation of measurements. However, it is probable that the number of measurements required for such purposes will often prove to be forbiddingly large.

II. THE CHROMOSOME COMPLEMENT OF MAN

The Human Karyogram. Tjio and Levan (1956) laid the foundation for a detailed study of the morphology of human chromosomes. Since then several such investigations have been published (Ford, Jacobs and Lajtha, 1958; Tjio and Puck, 1958; Chu and Giles, 1959; Lejeune, Turpin and Gautier, 1959; Böök, Fraccaro and Lindsten, 1959; Levan and Hsu, 1959). They are in substantial, if not complete, agreement on the major features of the chromosome complement although a different system of chromosome classification had been employed in each case. Perhaps this is a useful reminder that no such system has much biological meaning. However, a classification in which the chromosomes are arranged more or less according to size will in a rough manner reflect the number of genes in individual autosomes and thus have at least some genetic relevance.

It has become a practice to label each chromosome in a given cell with a number in the chosen system, even when the author obviously realizes that the assigned numbers hide a great deal of uncertainty. It is suggested that this practice be discontinued and that the labelling be carried only so far as the author feels confident of a correct identification. For the purpose of realistic labelling, the complement ought to be divided into chromosome groups that can readily be distinguished from one another in all cells which are at all suitable for an analysis. Only if an individual chromosome can be identified should the group symbol be replaced by the number of the chromosome.

With the above in mind, karyograms of the human complement were examined (Fig. 4). They virtually dictate the classification of the chromosomes into the groups A through G as shown in Figs. 4 and 5. This system of groups has already been used in studies of human trisomics (Patau, Smith, Therman, Inhorn, Wagner, 1960; Smith, Patau, Therman and Inhorn, 1960) and will be discussed in some detail in the following sections. A comparison of various published classifications with the present one (table 1) reveals the latter to be similar to the system of Ford, Jacobs and Lajtha (1958) and, except for the sex chromosomes, to be identical with that of Böök, Fraccaro, and Lindsten (1959). However, none of these authors have employed group symbols. Other authors have done so, but their systems are unacceptable to us because of a subdivision of the present C group. It will be pointed out below that no sub-group of C can be identified with any assurance in individual cells. Not surprisingly, there is no agreement between any two of the three published attempts at such a sub-division.

Of the above listed authors only Levan and Hsu (1959) do not regard all chromosomes of the present C group as being well separated from all other groups. They assign their No. 6 to the present B rather than to the C group. This seems odd as in a karyogram based on these authors' own figures (their table 3) No. 6 is 2.7 times closer to the nearest C chromosome than to the nearest B chromosome.

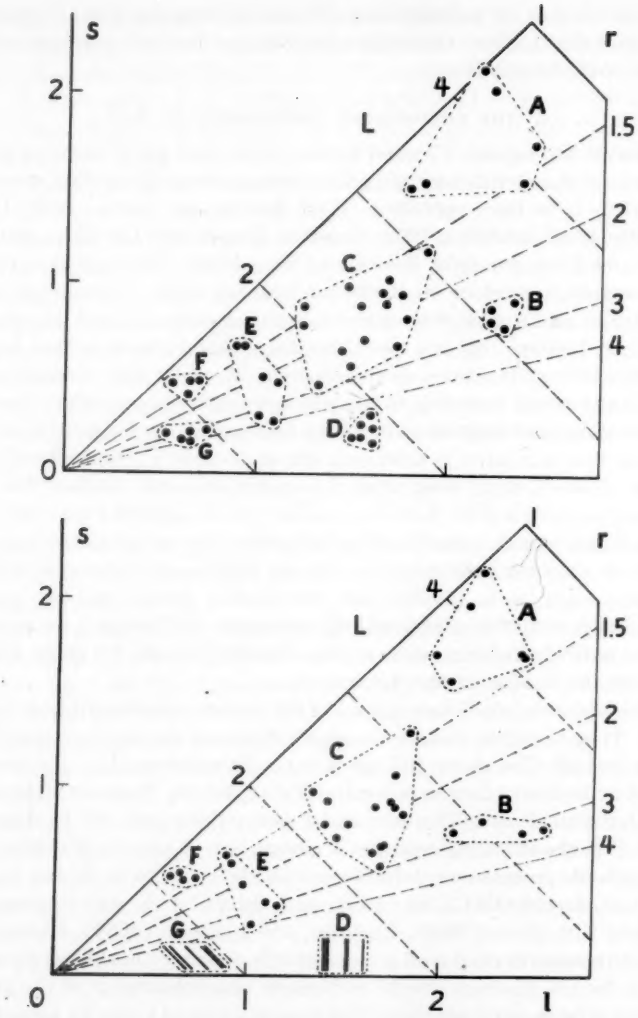

FIG. 4. Karyograms of human males. 1: length of long arm, s: – – short arm, L = 1 + s: chromosome length; all in percent of total length of complement. r = 1/s: arm ratio. Note: because of limited resolving power of microscope, the measured lengths of very short arms (especially in groups G and D) may differ greatly from true lengths. Top: the complement of Fig. 5. Bottom: the chromosomes of Fig. 1 by Lejeune et al. (1959) (of the G chromosomes only the total lengths, of the D chromosomes only the long arms were measured).

TABLE 1. KEY RELATING DIFFERENT CLASSIFICATIONS OF HUMAN CHROMOSOMES

Present Study	Book et al. (1959)	Ford et al. (1958)	Tjio & Puck (1958)	Chu & Giles (1959)	Lejeune et al. (1959)	Levan & Hsu (1959)
A 1 2 3	1 2 3	1 2 3	I 1 2 3	I 1 2 3	C_1 C_2 C_3	I 1 2 3
B 4 5	4 5	4 5	II 4 5	4 5	C_4 C_5	II 4 5 6
C X and 6-12	X and 6-12	6, X 8-13	III X 6	II X 6-8	M_1-M_4	III X 7-12
			IV 7-12	III 9-12	X M_{d1}-M_{d3}	
D 13-15	13-15	14-16	VII 18-20	IV 13-15	T_1-T_3	VI 18-20
E 16 17 18	16 17 18	19 17 18	V 13 14 15	V 16-18	P_1 P_2 C_1	IV 13 14 15
F 19 20	19 20	20 21	VI 16 17	VI 19 20	C_2 C_3	V 16 17
G 21 22 Y	21 22 Y	22 23 Y	VIII 21 22 Y	VII 21 22 Y	V_h V_s Y	VII 21 22 Y

It will be understood that in the present classification the numbers assigned to chromosomes which as yet cannot be identified are merely meant to be kept in reserve, as it were, until such an identification might become possible.

Groups A and B. The chromosomes Nos. 1, 2 and 3 are easily identified. The arm ratio of No. 1 has been reported to be 1.07, 1.08, or 1.1 (Tjio and Puck, 1958; Chu and Giles, 1959; Levan and Hsu, 1959), an inequality of the arm lengths which is also evident in karyograms but may, nevertheless, be largely artificial. The lengths of the arms of a chromosome seem to vary independently; and in the absence of a marker, such as a secondary constriction, there is no way of telling whether an arm that in the preparation is slightly longer than the other one has actually a larger true length or is a mere plus variant. If an unmarked chromosome should have the true arm ratio $r = 1$, its distribution in the karyogram would still fall below the 45° line and the mean of the measured arm ratios would be larger than one. A good example is provided by the two satellite chromosomes IX of the rhesus monkey in Fig. 2. If there had been no marker, both chromosomes would have been placed below the 45° line and their mean arm ratio would have been $r = 1.17$ instead of $r = 1.08$.

In cases like that of chromosome No. 1 in man, an unbiassed estimate of the true arm artio could be obtained by a statistical analysis of the distribution of arm lengths determined for a sufficiently large number of homologous chromosomes. It would have to be assumed that $x = \log r$ is normally distributed around the logarithm of the true value of r. This assumption is no doubt valid for all practical purposes. The, solvable, problem then would be to estimate the parameters of a normal distribution, $\phi(x)$ in Fig. 6, so that the distribution $\Psi(x) = \phi(x) + \phi(-x)$, for $x \geq 0$, fits the observed distribution of $x = \log r$. Such an analysis may become necessary if measurements of a translocation involving chromosomes No. 1 should have to be evaluated.

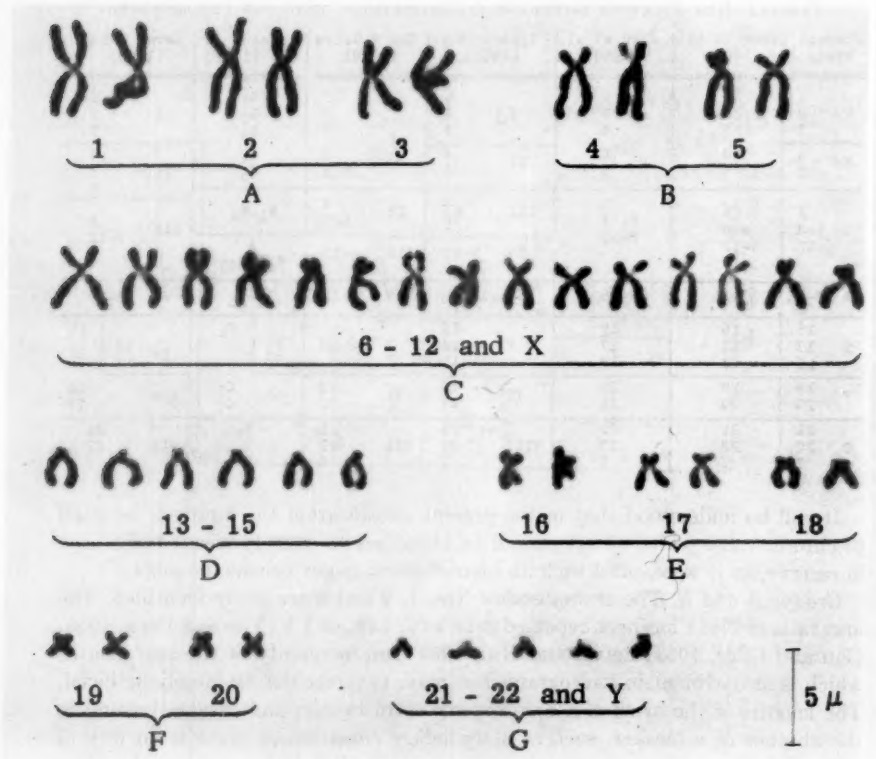

FIG. 5. Chromosome complement of human male (bone marrow fixed in acetic alcohol 1:3, fresh Feulgen-Orcein squash). The 5th G chromosome is probably the Y.

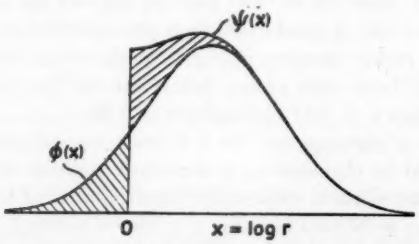

FIG. 6. True ($\phi(x)$) and apparent ($\psi(x)$) distribution of arm ratios of chromosome with nearly median centromere. See text.

The chromosomes Nos. 4 and 5 have been set apart in a group of their own—which indeed stands out in any karyogram—because these pairs cannot always be distinguished reliably from one another. For this reason it is desirable to have a group symbol, B, for designating these four chromosomes when analyzing a

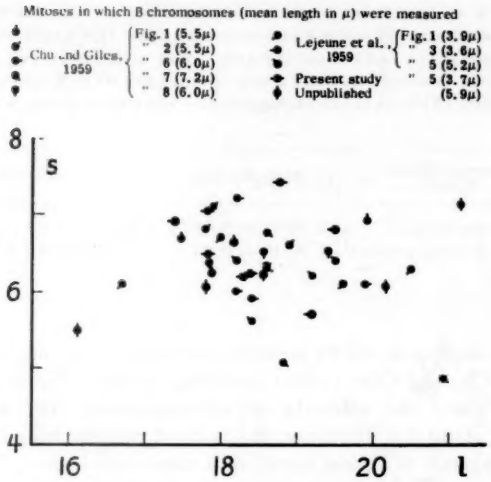

FIG. 7. Ten partial karyograms of B group superimposed. l: length of long arm, s: - - short arm, both in percent of group total.

complement. The partial karyogram of Fig. 7 shows that the distributions of Nos. 4 and 5 overlap considerably and that many more measurements will be needed before an attempt can be made to estimate the true lengths of these chromosomes by statistical means. There seems to be no appreciable difference in the lengths of the short arms. The long arms of the B chromosomes provide an example for the fact that in certain cases chromosomes can be identified in individual cells more reliably by a direct comparison than by length measurements. For instance, the latter are inconclusive for the B group in Figs. 5 and 4, top, as will be evident when the small length differences between these chromosomes are related to the length variation manifest in Fig. 7. Nevertheless, the distinction between Nos. 4 and 5 seems to be feasible, for both under the microscope and in Fig. 5 the long arms of the chromosomes No. 4 appear to be somewhat heavier than those of No. 5. Altogether the observations indicate that the difference in true length of the long arms is such that in cases in which two pairs happen to be well separated in the karyogram (see Fig. 4, bottom) the members of each can be relied upon to be homologous. The mean lengths of such pairs are, however, obviously not unbiassed estimates of the true lengths.

It has been claimed that colchicine treatment has a distorting effect upon the length of chromosome arms (cf. Lejeune et al., 1959). A statistical test of the data represented in Fig. 7 does not support this idea (Table II). Neither the arm ratio nor the variance of the chromosome length appear to be appreciably affected by a treatment with colchicine. There seems to be no reason as yet to forsake the advantages of this treatment.

Group C. This group contains the X chromosome and seven pairs of autosomes. The two C groups in the karyograms of Fig. 4 might seem to indicate that the longest chromosome of the group is an odd one and, therefore, the X chromo-

TABLE 2. TEST FOR AN EFFECT OF COLCHICINE TREATMENT ON THE ARM RATIO AND ON THE LENGTH VARIATION OF THE FOUR B CHROMOSOMES. THE ARM RATIO WAS COMPUTED PER NUCLEUS AS THE TOTAL LENGTH OF THE LONG ARMS DIVIDED BY THE TOTAL LENGTH OF THE SHORT ARMS. FOR EACH MITOSIS A SUM OF SQUARES WITH 3 D.F. WAS DERIVED FROM THE CHROMOSOME LENGTHS GIVEN IN PER CENT OF THE GROUP TOTAL. SAME DATA AS USED IN FIG. 7.

Colchicine Treatment	Number of Mitoses (Source)	Mean Arm Ratio	Mean Sum of Squares
No	3 (Lejeune et al.)	2.98 $t_s = 0.46$	1.470 (9 d.f.) $F = 1.23$ not significant
Yes	7 (Chu & Giles, present study)	2.92 $P = 0.66$	1.813 (21 d.f.)

some. If so, the finding would be incompatible with the claim by Tjio and Puck (1958) and by Chu and Giles (1959) according to whom the X chromosome has an arm artio of about two, while the odd chromosome in Fig. 4 has in both cases an arm artio of about 1.5. However, the isolated position of one chromosome in these two karyograms is almost certainly a mere coincidence. The chromosome in question in Fig. 4, top, is shown as the first in the C group of Fig. 5. It could well be homologous to the second C chromosome in this Fig., as the latter chromosome is not only shorter but also somewhat thicker, which could be seen under the microscope as well as in Fig. 5. Furthermore, and this is the decisive point, most of the partial karyograms of the male C group (Fig. 8) do not show an odd chromosome at this location. Nor do the partial karyograms of the female C group (Fig. 8) indicate pairs of X chromosomes at this location.

A comparison of the male-and female C groups in Fig. 8 fails to reveal any consistent separation into sub-groups or any consistent suggestion of an X chromosome occurring singly in the male and as a pair in the female. Obviously, it is meaningless to try an isolation of the X chromosome in these clusters of points by any pairing-off procedure. Insofar as all previous attempts to identify the X were based on some variation of the pairing-off method one can but conclude that the location of this chromosome within the C group is as yet unknown. The published claims, incidentally, are in part contradictory. Tjio and Puck (1958) and Chu and Giles (1959) describe the X as the largest of the C chromosomes and as having an arm ratio of about two. However, the latter authors illustrate among their presumed X chromosomes three (in their Fig. 3) which have a much smaller arm ratio. Böök et al. (1959) put the X chromosome tentatively between their chromosomes No. 6, which is their largest C chromosome, and No. 7. Lejeune et al. (1959) assign the X to the shorter chromosomes of the C group. Levan and Hsu (1959) label the largest chromosome of their group III as the X but add ". . . in our opinion the identification of the X in somatic metaphases cannot be considered definitive as yet, for any of the other chromosomes of group III is just as eligible."

An attempt was made to estimate the location of the X chromosome in the C group by means of the statistical methods discussed in the first part of this

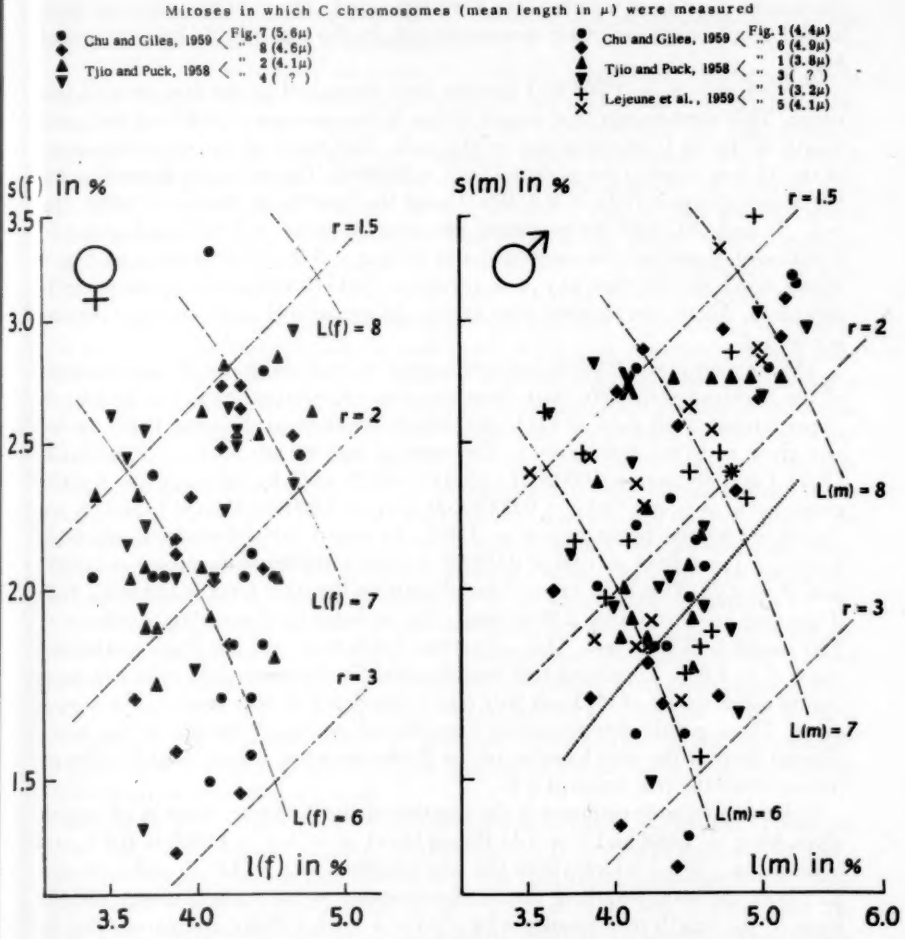

FIG. 8. Partial karyograms of C group superimposed. All lengths in percent of group total. Logarithmic scales. X: computed estimate of X chromosome; dotted line: lower fiducial limit of X. See text.

study. The C chromosomes were measured in photomicrographs published by other authors. It will be evident that the $N_m = 6$ male and $N_f = 4$ female cells have been selected exclusively for technical suitability. The lengths of both chromatids of each chromosome arm were determined separately, except in cases of very close association, and averaged. The measurements were done carefully by applying a millimeter ruler to the intervals between points along the chromatid axis, the points being the closer spaced the stronger the curvature of the

chromatid. Tenths of a millimeter were estimated. The resulting data are presented graphically, and their sources stated, in the logarithmic karyograms of Fig. 8.

The estimate q = 1.068 had already been computed in the first part of this paper. This corresponds to a length of the X chromosome of 6.8% of the total length of the 15 C chromosomes in the male. The mean of the six group totals of the 15 long arms in the male is $\overline{l_t(m)} = 0.66087$. The corresponding value for the female group is $\overline{l_t(f)} = 0.65108$. Under the hypothesis that q = 1.068, the eqs. (5) and (3) yield the estimated arm lengths $l_x(m) = 0.0345$ and $s_x(m) = 0.0335$ and, therefore, the estimated arm ratio r = 1.03. This value is, as Fig. 9 shows, much smaller than any encountered in the C chromosomes of the ten cells measured. About the smallest true arm ratio compatible with the observations is r = 1.5.

The statistic r = 1.03 definitely represents an underestimate of the arm ratio of the X chromosome. We need, therefore, not a fiducial interval, but merely an upper fiducial limit for r or for $l_x(m)$, which amounts to the same thing as, for any given q, $l_x(m)$ determines r. The sums of squares are SS(f) = 0.00055151 (3 d.f.) and SS(m) = 0.00028171 (5 d.f.) which yield by means of (6) for the given value of q: $s(l_x(m)) = 0.00689$. It is $t_8 = 1.860$ for P = 0.1. Hence, we obtain, under the hypothesis q = 1.068, the upper fiducial limit on the 95% level $l_x^*(m) = 0.0345 + 1.86 \times 0.00689 = 0.0473$ which implies $s_x(m) = 0.0207$ and $r^* = 2.28$. This is an upper fiducial limit on the 95% level in the sense that if the true arm ratio were 2.28 or larger the probability of sampling a value $r \leq 1.03$ would be 5% or less. This, of course, holds true only for the hypothetical value q = 1.068. Repeating this computation for other possible values of q we obtain other values of r^*. Each pair (q, r^*) corresponds to a point in the karyogram. These points determine the line entered in Fig. 8 (male) as the lower fiducial limit of the true location of the X chromosome, a limit that is valid no matter what the true value of q is.

The best available estimate of the location of the X chromosome is, of course, given by q = 1.068 and r = 1.5. Hence $l_x(m) + s_x(m) = 0.068 = 6.8\%$ and $l_x(m)/s_x(m) = 1.5$ which yields the arm lengths $l_x(m) = 4.08\%$ and $s_x(m) = 2.72\%$ of the total length of the 15 chromosomes of the male C group. On the basis of the length measurements by Tjio and Puck (1958), the value 6.8% of the male C group can readily be shown to be equivalent to 4.8% of the total length of the haploid female complement. (As the X chromosome is not recognizable the total haploid female complement is a more precise reference quantity than the autosomal total used by other authors.) In view of the inherent uncertainty of these estimates, which could be greatly reduced only by an analysis of a large number of additional measurements, it is fortunate that their plausibility can be subjected to an independent test. If the estimated location of the X chromosome is approximately correct we should expect that in the neighborhood of this point the karyogram of the female C group shows a higher density than that of the male C group. There is nothing in the computation of q and r which would automatically ensure such an excess of chromosomes at this particu-

lar location. The following represents, therefore, an independent test of the estimated location of the X chromosome.

The first step is to shift the points (x,y) of the logarithmic karyogram of the female C group so that their new co-ordinates are x + c and y + c, c being the logarithm of q = 1.068. Next, the same grid, as before with the unit 0.025, is superimposed on the male and on the shifted female karyogram. Then, for each point of the grid in either karyogram those chromosome points are counted that lie within a surrounding circle of the size shown in Fig. 9. This area is chosen large enough to make it likely that all or most of the points which represent chromosomes with a true location close to the center of this circle will be contained within the circle. All counts obtained from the male karyogram are multiplied by $\frac{2}{3}$ to compensate for the difference between the sexes in the number of cells analyzed. Subtracting, for each point of the grid, the thus adjusted male count from the female count we obtain a chart of differences the main features of which are represented in Fig. 9. If all chromosomes were present in their true lengths all differences would be naught except near the location of the X chromosome where a difference of +4 could be expected. The, in fact, very considerable length variation is bound to produce in this diagram local excesses and deficits of the female relative to the male count that are unrelated to the presence of the additional four X chromosomes in the female C group. Nevertheless, the most likely locations of the X are obviously areas of excess. Therefore, the previously estimated location of the X chromosome is to some extent confirmed by the fact that in Fig. 9 this location is well within the one area that has a marked excess of chromosomes in the female. Considering that the deficits in other areas must be compensated by part of this excess, most likely from areas closest to the areas of deficit, it appears indeed that the computed arm lengths of the X chromosome fit Fig. 9 almost perfectly. Even so, these lengths should be regarded only as first tentative estimates. The analysis of future measurements will no doubt lead to more definite ones.

Group D. The chromosomes of this group can be easily recognized. There are three very similar pairs of them, all acrocentric. It is certain that not all have the same length, for one can always find two that differ unmistakably in their amount of Feulgen positive material. However, it is no less evident that the true length differences are not large enough, relative to the variation within pairs, to render a pairing-off by length meaningful. Similarly, there are no doubt differences in the size of the short arm but this arm is so variable in appearance, depending on how the chromatids and the centromere happen to lie, that it, too, can probably not be used to pair off homologues. However, Lejeune et al. (1959) claim that Unna's blue brings out "petits bras hétérochromatiques" in two of the D pairs while the third pair has short arms that are "nettements plus courts." What criterion would justify to call these very small structures, as seen in metaphase, heterochromatic is not explained.

The chromosomes of one pair (presumably of only one pair, although the available observations do not suffice to prove this point) have a minute satellite (Tjio and Puck, 1958; Chu and Giles, 1959; Böök et al., 1959; Levan and Hsu,

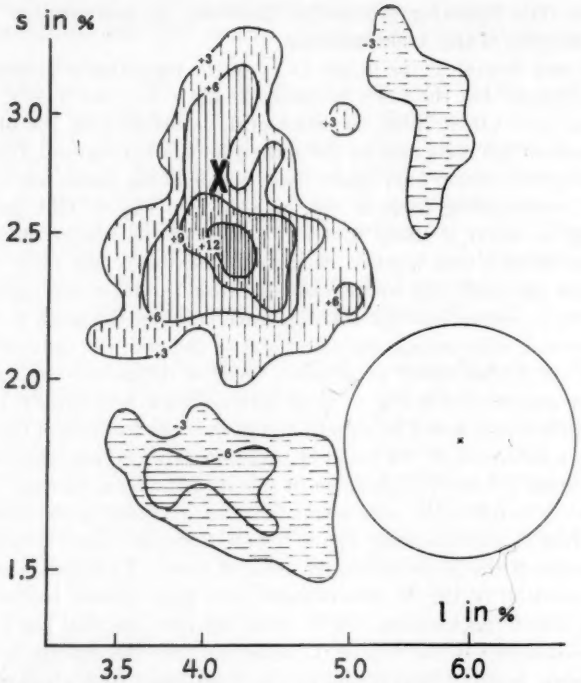

FIG. 9. Analysis of C group (see text). Vertically shaded: areas of points for which surrounding circle of indicated size contains an excess of at least three chromosomes in four female cells, relative to male cells, after shift by c = log 1.068. Horizontally shaded: correspondingly defined areas of deficit. X: computed estimate of X chromosome.

1959). It can be seen readily enough in orcein slides, less well in Feulgen slides, but unfortunately not very regularly. Therefore, it has so far been impossible to decide whether the extra D chromosome in the case of trisomy reported by Patau et al. (1960) has or has not a satellite.

Group E. The three pairs of this group can definitely be distinguished from each other in favorable cells. Patau, Smith, Therman, Inhorn and Wagner (1960) arranged these chromosomes in a sequence identical with that used by Ford et al. (1958) even though this meant putting one chromosome behind two which are somewhat smaller. Since then the paper by Böök et al. (1959) has reached us in which a system of numbering human chromosomes is employed which agrees with the one we had adopted except for the E chromosomes. We shall henceforth follow the numbering system of these authors throughout. This brings the E chromosomes into the sequence first used by Tjio and Puck (1958). No. 16 has a submedian centromere, No. 17 is a little shorter and has a subterminal centromere, No. 18 is still shorter and its short arm is so small that this chromosome could almost be called acrocentric. Indeed, it could sometimes be mistaken

for a D chromosome if it were not for its appreciably smaller size. If the E chromosomes are not lying horizontally with their chromatids side by side and uncrossed, No. 18 can be confused with No. 17 and even No. 17 with No. 16. In the cases of trisomy for an E chromosome described by Smith, Patau, Therman and Inhorn (1960) we found it impossible to decide which of the E chromosomes was present in triplicate. In subsequently discovered cases of the same trisomy syndrome the extra chromosome could be identified as No. 18.

Groups F and G. The F group contains two pairs of autosomes with very nearly median centromeres. These chromosomes are very similar to each other and quite distinct from all other chromosomes. The same holds true for the two pairs of acrocentric autosomes in the G group to which, in the male, also the Y chromosome belongs. The arm lengths in both groups are of the order of one mieron with the exception of the short arms of the G chromosomes, which are much shorter. Under these circumstances measurements of the arm lengths depend greatly on a consistent interpretation of diffraction fringes. However, no such consistency can be expected if the chromosomes to be compared do not have the same width of the centromere gap, if their chromatids are not lying perfectly horizontally, or do not form a very similar pattern. These ideal conditions are seldom adequately approximated. The usefulness of length measurements for the purpose of identifying these small chromosomes is, therefore, open to even graver doubts than it is in the case of longer chromosomes. The human eye, on the other hand, can make allowances for some variation in chromosome contraction and in width of the centromere gap, and even for a moderate upward or downward bend of a chromosome arm. Thus, relatively small differences in chromosome mass can probably be assessed more reliably by direct observation than by measurement. Such differences are rendered more strikingly in photomicrographs that had been processed for high contrast, but this approach is fraught with too many sources of error to be relied upon. The documentary value of high power photomicrographs is not always beyond question.

It seems to be true that in cells in which the chromosomes of the F group are favorably displayed the members of the pair No. 19 can be identified by a slightly larger size (see Fig. 5). The same can apparently be said for the pair No. 21 of the female G group, leaving aside for the moment the male G group in which the presence of the Y complicates matters. It is the author's impression that the size difference between the two autosomal G pairs is at least in part due to No. 21 having a larger short arm. Lejeune et al. (1959) report that after staining with Unna's blue "petits bras hétérochromatiques" are seen in one pair of G chromosomes, the other one "n'en ayent pas ou practiquement pas." This would seem to be in substantial agreement with the view expressed here, if from the last quoted phrase the words "pas ou" and from the first "hétérochromatiques" were eliminated. All G chromosomes definitely have short arms, however small, and the meaning of heterochromasy at metaphase is certainly doubtful.

The same authors who described the satellite at a D chromosome have also reported satellites in the G group. Most, including Chu and Giles (1959), believe that the satellite chromosomes are the larger ones, i.e. those of pair No. 21.

The photomicrographs published so far are hardly conclusive in this respect. However, in Fig. 8 of Chu and Giles (1959) the two satellited G chromosomes appear to have distinctly smaller short arms than the non-satellited one (the fourth G chromosome is partially hidden), in other words, the satellites seem to belong to No. 22. This is also the conclusion which Levan and Hsu (1959) and, independently, the present author have drawn from their own findings. The latter has seen satellites repeatedly at chromosomes that had to be identified as No. 22 because of their minute short arms and of a total mass which seemed to be clearly smaller than that of the other pair of G autosomes. Levan and Hsu's Fig. 2, in particular the upper plate, illustrates this situation very well.

The observations by Lejeune et al. (1959) and by Böök et al. (1959) probably mean that the G chromosome which causes mongolism when present in triplicate can by its size be identified as No. 21. If so, the mongolism chromosome should in the present writer's view not have a satellite; yet Böök et al. (1959) found in some cells of mongoloids three G chromosomes "with satellite-like formations" and this is what their Fig. 10 shows. However, the presumed satellite of one of the three chromosomes appears to be so very much smaller than the other ones that one hesitates to accept this as final evidence. Besides, it is of course a mere presumption, though a reasonable one, that only one of the G pairs has satellites.

The Y chromosome is in the author's opinion slightly larger than the autosomes of the G group. The impression prevails that in male cells in which all G chromosomes are well displayed there is one odd chromosome that is not seen in comparable female cells. This presumed Y chromosome may appear similar to the other G chromosomes but slightly larger and with a relatively large short arm (see the last chromosome in the G group of Fig. 5). In other cells, and then most convincingly, it is seen as consisting of two more or less straight chromatids, quite often without a visible centromere. The latter, however, is evident as a fine gap in the presumed Y of Fig. 10. This type of G chromosome does not seem to occur in female cells.

The views concerning the identity of the Y chromosome that have been expressed in recent publications are far from unanimous. Ford, Jones, Miller, Mittwoch, Penrose, Ridler and Shapiro (1959) let the matter rest with the remark that the Y chromosome, "though very similar, is usually distinguishable from" the other G chromosomes. Their Fig. 2 suggests that their identification is identical with the one proposed above. This may also be true for the chromo-

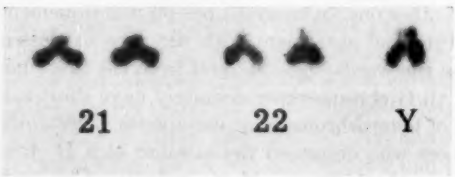

FIG. 10. Tentative identification of chromosomes in male G group (bone marrow). 5020X.

some which Tjio and Puck (1958) consider to be the Y even though the present author cannot agree with their claim that the latter has "a terminal or almost terminal centromere, as opposed to the distinct short arms possessed by the two autosome pairs of this group." Levan and Hsu (1959), too, describe the Y as the largest G chromosome with a short arm that appears distinctly smaller in the drawing of their Fig. 2 than the short arm of the presumed Y in the present Fig. 10.

On genetical grounds it appears, of course, possible that small deficiencies in the Y chromosome cause so little selective disadvantage as to allow an appreciable degree of Y polymorphism to persist in human populations. Indeed, the present author has the impression that the Y does not stand out equally well in the mitoses of all males. However, critical evidence for such a polymorphism would not be easy to obtain. At least most of the contradictory descriptions of the Y in the current literature are probably due to mistaken identifications rather than to polymorphism.

Chu and Giles (1959) regard the Y as being "apparently" the smallest chromosome in the complement. However, their Fig. 6 shows a typical example of the chromosome discussed above as being most likely the Y. It is the lowest of the two chromosomes labelled "22." The homology of these chromosomes is questionable to say the least. They do have about the same length but seem to differ greatly in total mass. The chromosome that in the present author's opinion is the Y displays its two chromatids side by side. If these were lying one on top of the other, they might form a mass similar in shape to the other chromosome "22" but this mass would no doubt appear much denser than the latter, as can be seen from other chromosomes, in the same photograph, in which chromatids overlap. The present writer interprets the smaller chromosome "22" as being homologous to one of the other G chromosomes but with its long arms pointing in opposite directions away from the centromere that is hidden beneath the short arms, which cause the higher density of this chromosome in its middle.

Judged by their Fig. 6, Böök et al. (1959) also seem to consider the Y to be the smallest chromosome of the complement. However, both in this and in their Fig. 2 a typical representative of the Y chromosomes as characterized above can be seen. It is in each case the rightmost of the chromosomes labelled "21."

It appears thus that the tentative identification of the G chromosomes, especially of the Y, as given in Fig. 10 is by and large compatible with the evidence presented by other authors, though not always with their interpretations. While the present writer believes that the G chromosomes of Fig. 10 are labelled correctly, he does not feel that the issue should be closed. The fact that for many years a human chromosome number of 48 had generally been taken for granted should warn us not to acquiesce too readily in conclusions, however plausible, that have not yet been amply demonstrated to be necessary conclusions.

SUMMARY

The use of length measurements of chromosome arms for the purpose of identifying homologues is examined from a methodological view point. The pairing-

off by length, as frequently done, is likely to be grossly misleading. Graphical and statistical methods for the evaluation of length measurements are discussed.

The chromosome complement of man is reviewed critically (for its proposed classification see Figs. 5 and 10). The claim that every pair of homologues can be identified cannot be sustained. In particular, the X chromosome cannot be distinguished from similar-sized autosomes.

A first attempt to determine the length, L, and the arm ratio, r, of the X chromosome by a statistical evaluation of length measurements yields the, still very tentative, estimates L = 4.8% of the haploid female complement, and r = 1.5.

ADDENDUM

Since the present manuscript went to press, the conclusions of the Denver "Study Group" have become known.[1] The author was gratified to learn that both the numbering system of chromosomes and the system of groups adopted in Denver are identical with the classification used in the present work, at least within the limits of identifiability as the present author continues to see them. He also continues to believe that the X chromosome cannot yet be identified with any assurance among the C chromosomes, or, to use the Denver terminology, among the chromosomes of the group 6-12. This point is borne out by the very same data which the Denver group have provided in their table 2, when these data are plotted as karyograms (Figure 11). Karyograms can, of course, be based just as well on the total chromosome length and the arm ratio as on the arm lengths. If the locations of the X chromosome relative to that of the autosomes in these karyograms are compared with each other, it will become evident that there are such contradictions between the various identifications of the X chromosome that at least some of the authors must have been mistaken. The discrepancies are by far too large to be explained by differences in the techniques of preparation and measurement. In view of these differences of opinion about the identity of the X chromosome, differences which the members of the Denver Study Group perhaps did not fully realize, their recommendation that in karyotypes the sex chromosomes be arranged "near to but separated from the autosomes they resemble" appears not very practical.

The six groups of authors who have contributed to table II of the Denver Report seem to agree quite well about the characteristics of chromosome No. 6. However, when these data are related to the chracteristics of the X chromosome as proposed by the various authors, even the identification of No. 6 would seem to be spurious. The data concerning the remaining autosomes of the C group in the same table II are even more contradictory. It will further be noticed that in this table chromosome No. 15 is assigned the smallest arm ratio within the D group once, an intermediate arm ratio twice, and the largest arm ratio three times.

It should perhaps be pointed out that the question whether a cytologist can identify the X chromosome within the C group is not as hopelessly subjective as

[1] "A proposed standard system of nomenclature of human mitotic chromosomes". *Eugenics Quart.*, 7: 96–100 (1960) also *Am. J. Human Genet.*, this issue.

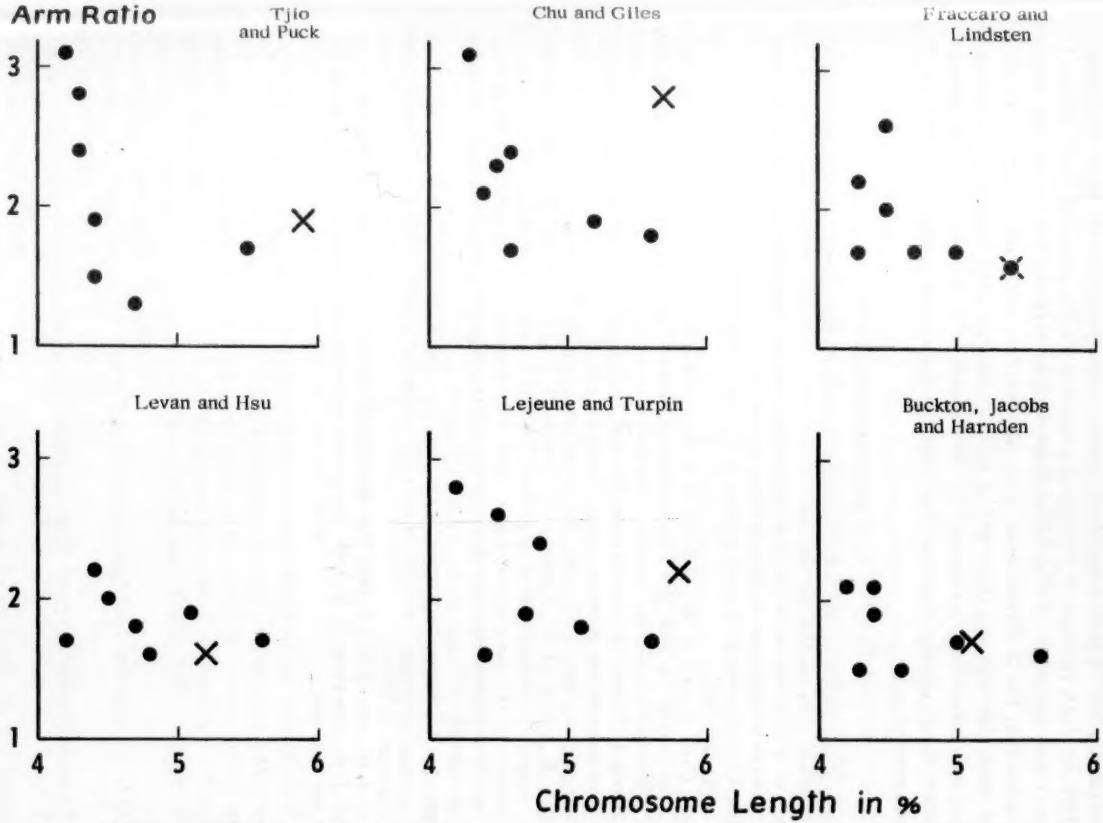

Figure 11. Karyograms of the haploid C group, representing the mean values A and B as given in Table II of the report of the Denver Study Group. Circles: autosomes Nos. 6–12, cross: X chromosome.

it might seem to be. If from photomicrographs of a sufficient number of very favorable plates of both sexes the C chromosomes were cut out, to be kept separately for each nucleus, if further the smallest chromosome of each female C group were removed (this could be done without detriment as everybody seems to agree that the X chromosome is not the smallest one), and if then the cytologist could correctly sex these sets of 15 chromosomes each (which he should not have seen before the cutting-out) by the number of X chromosomes, the present author would gladly concede that this cytologist can indeed identify the X chromosome.

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Studies on Linkage Between Phenylketonuria and the Blood Groups[1]

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PENROSE AND OTHERS (Munro, Penrose, and Taylor, 1939, Penrose, 1945 and 1951) reported data which suggested that loose linkage between phenylketonuria and the ABO locus may exist. The data were too few to permit a statistically valid conclusion to be reached. The difficulty of accumulating the large samples necessary to test loose linkage for a recessive character rigorously, prevented these authors from bringing the analysis to a statistically significant conclusion.

In 1956 Hsia, Driscoll, Troll, and Knox reported that the heterozygous carriers for phenylketonuria can be detected by means of a phenylalanine tolerance test. A load of 0.1 gm. per kg. body weight of L-phenylalanine was administered by mouth, and samples of blood were taken at one, two, and four hours, and the plasma phenylalanine levels determined. It was found that the heterozygotes had plasma levels on the average twice that seen among normal controls and the difference between the two groups was highly significant. The ability to detect the heterozygote combined with newly reported methods for the detection of linkage in man (Morton, 1955) made it seem worth while to reinvestigate the problem.

The purpose of the present paper is to report on linkage relationships between the genes for phenylketonuria and those for the blood groups. The data have been subjected to sequential analysis with the phenylketonuric gene being treated both as a recessive and as an intermediate dominant, where the heterozygote is identified by means of the phenylalanine tolerance test.

MATERIALS AND METHODS

A total of 23 families located in New England and the Midwest were studied. In each instance, the diagnosis of phenylketonuria in the homozygote was confirmed by the presence of phenylpyruvic acid in the urine or the elevation of phenylalanine in the plasma, or both. With one exception, which has been reported in a separate communication (Hsia, Knox, and Paine, 1957), all of the homozygotes were mentally deficient.

Venous blood samples were tested for the following blood groups: (1) ABO (2) MNS (3) Rh (4) P (5) K and (6) Fy.

Available relatives (as shown in Appendix Table A) were subjected to a stand-

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ard phenylalanine tolerance test (Hsia et al., 1956). One-tenth of a gram per kg. of L-phenylalanine (obtained from Nutritional Biochemicals, Cleveland, Ohio) in crystalline form was mixed with fruit juice and given by mouth after an overnight fast. Samples of plasma were taken one and two hours after the load and the phenylalanine level determined by the phenylethylamine method of Udenfriend and Cooper (1953) as modified by Hsia, Knox, Quinn, and Paine (1957). The plasma phenylalanine levels at one and two hours were summed and used to establish the discriminant between heterozygotes and normal controls.

The tolerance test to detect heterozygotes for phenylketonuria (Hsia et al., 1956) is not completely successful (Hsia, 1958). Regardless of the score used (phenylalanine-tyrosin ratio, the sum of the one- and two-hour phenylalanine levels, etc.) there is considerable overlap of the scores for known heterozygotes and presumed homozygous normals. An example of this is shown in Fig. 1, where the logarithms of the sums of the one- and two-hour phenylalanine levels in mgms % for 48 heterozygotes (parents of affected children) and 38 presumed normals are plotted. (Unpublished calculations showed that for our data this score was as satisfactory as any other for discriminating between homozygotes and heterozygotes.) We were confronted with the alternatives of treating phenylketonuria as a complete recessive, or of using some arbitrary criteria for deciding whether an individual was homozygous or heterozygous. We decided to utilize both alternatives.

The heterozygosity of a nonphenylketonuric member of a family was determined as follows: 1. Normal curves were fitted to the distributions of the

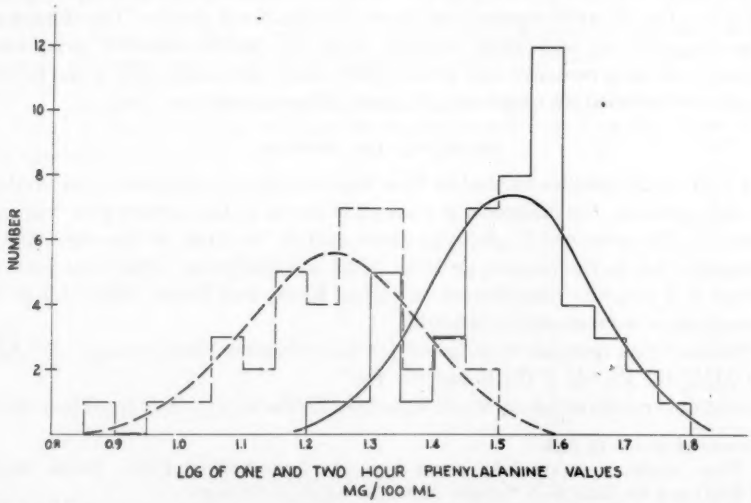

FIG. 1. Histograms and fitted normal curves for the distribution of the sums of the one- and two-hour phenylalanine levels in 38 normal adults (dashed lines) and 48 heterozygotes (solid lines).

logarithms of the sum of the one- and two-hour phenylalanine levels for known heterozygotes and for presumed normals. 2. When it was ascertained that the fitted normal curves were satisfactory representations of these distributions, deviations from the means were computed, taking into account the prior odds (segregation ratios), so that the frequency, at these deviations, of homozygous normals would be to that of heterozygotes as 4:1 or as 1:4. Values falling at or to the left of the former point were considered to be for homozygous normals; those at or to the right of the latter point were considered to be for heterozygotes. Values falling between these points were classified as indeterminate and not used for linkage analyses. By this procedure the odds were at least 4:1 that the classification of each individual was correct.

The decision points were determined by equating the ratio of the ordinate of the normal curve fitted to the distribution of the sum of the one and two hour plasma phenylalanine levels of the homozygotes, to the ordinate fitted to that of the heterozygotes, at 4 for the upper limits for normals and at 0.25 for the lower limit for heterozygotes, and solving for the value of the sum of the one and two hour levels which would satisfy these values of the equation.

The equation is:

$$\frac{y_1}{y_2} = \frac{\dfrac{N_1}{\sigma_1\sqrt{2\pi}} \exp - \dfrac{(x_i - \bar{X}_1)^2}{2\sigma_1^2}}{\dfrac{N_2}{\sigma_2\sqrt{2\pi}} \exp - \dfrac{(x_i - \bar{X}_2)^2}{2\sigma_2^2}} = Y$$

where y_i = ordinate of normal curve

N_1 = the *a priori* frequency of homozygous normals relative to that of heterozygotes

N_2 = the *a priori* frequency of heterozygotes relative to that of homozygous normals

x_i = logarithm of the sum of the one- and two-hour phenylalanine levels at a decision point

$\bar{X}$ = mean

σ = standard deviation

The subscripts refer to homozygous normals (1) and to heterozygotes (2).

The relative frequencies of homozygotes and heterozygotes are 1:1 for matings of Phph × PhPh and 1:2 for matings of Phph × Phph. Using the values and the computed means and standard deviations, the following "cut-off" points in mgms % for the sum of the one- and two-hour phenylalanine levels were established:

Mating	x_i at Y of Indicated Value	
	Y = 4	Y = 0.25
1. Phph × PhPh	20.3	29.1
2. Phph × Phph	18.5	25.9

Thus for cross 1 all those whose scores were 20.3 mgms % or lower were con-

sidered homozygous normal; those whose scores were 29.1 mgms % or higher were considered heterozygous; all others were excluded from consideration. Similarly, for cross 2 all those whose scores were 18.5 mgms % or lower were considered homozygous normal; those whose scores were 25.9 mgms % or higher were considered heterozygous; all others were excluded from consideration.

The family data upon which the linkage caculations are based are presented in Appendix Table A. Since only two of the parents and one of the siblings tested had weights exceeding 90 kilograms, the heights and weights of the individuals are not recorded in the table, nor were they considered in determining heterozygotes (Renwick, Lawler, and Cowie, 1960).

Scores for the sequential test for linkage (Morton, 1955) were computed for the ABO, MNS, Rh, Fy, K, and P loci, treating phenylketonuria as an intermediate dominant and as a recessive. We have in addition computed the linkage scores for the MN, and Rh loci for the families reported by Munroe (1947) and by Penrose (1951), treating phenylketonuria as a recessive. The totals for the scores are presented in Tables 1 and 2. Renwick et al. (1960) have examined the ABO data in detail.

When phenylketonuria is treated as an intermediate dominant (Table 1), the following conclusions may be drawn: ABO: linkage of .1 or less is excluded and probably also linkage of .2; MNS and Rh: linkage of .1 or less is excluded; K: linkage of .05 or less is excluded. No conclusions may be drawn concerning looser linkages than those mentioned above for these loci, nor for any linkage (except absolute linkage which is excluded) concerning the Fy and P loci.

TABLE 1. SEQUENTIAL ANALYSIS SCORES FOR LINKAGE BETWEEN PHENYLKETONURIA AND VARIOUS BLOOD GROUP LOCI. PHENYLKETONURIA TREATED AS AN INTERMEDIATE DOMINANT[1]

Blood Groups	No. of Families	z at Specified Values of θ				
		.05	.10	.20	.30	.40
ABO	12	−7.6839[2]	−4.5465	−1.9450	−.7061	−.0455
MNS	15	−7.0652[3]	−3.8228	−1.1862	−.2749	+.1717
Rh	16	−6.2933	−3.3098	−.9829	−.1651	−.0650
Fy	4	−.6807[4]	−.5104	−.1038	+.0074	+.0361
K	5	−2.3537	−1.3516	−.5307	−.1908	−.0424
P	5	−.1912	−.0112	+.0666	+.0476	+.0144

[1] Based on determination of the heterozygote by means of the phenylalanine stress test. Those for whom the *a priori* expectation of heterozygosity was ½ and for whom the sum of the 1- and 2-hour plasma phenylalanine levels was less than 20.3 mgm/100 ml., were considered homozygous normal; if the sum exceeded 29.1 mgm/100 ml., they were considered heterozygotes. All others were excluded from consideration. Those for whom the *a priori* expectation of heterozygosity was ⅔ were considered homozygous normal if the sum of the 1- and 2-hour readings was less than 18.5 mgm/100 ml.; they were considered heterozygous if the sum exceeded 25.9 mgm/100 ml. All others were excluded from consideration. Parents of affected children were accepted as heterozygotes regardless of their scores.

[2] Based on 11 families.

[3] Based in 12 families.

[4] Based on 3 families.

TABLE 2. SEQUENTIAL ANALYSIS SCORES FOR LINKAGE BETWEEN PHENYLKETONURIA AND VARIOUS BLOOD GROUP LOCI. PHENYLKETONURIA TREATED AS A RECESSIVE

Blood Groups	Source	No. of Families	z at Specified Values of θ				
			.05	.10	.20	.30	.40
ABO	This study	14	−1.9539	−1.0060	−.3065	−.0803	−.0128
MNS	This study	18	−3.8833[1]	−2.0976	−.7709	−.2607	−.0536
	Munro (1947)	16	—	−1.1207	−.0844	+.1076	+.0505
	Penrose (1951)	2	—	+.0793	+.0540	+.0275	+.0075
	The 3 studies	36	−3.8833[1]	−3.1390	−.8013	−.1256	−.1212
Rh	This study	19	−1.9938[2]	−1.7446	−.6425	−.2133	−.0436
	Penrose (1951)	5	−.8744	−.5294	−.2098	−.0723	−.0152
	The 2 studies	24	−2.8682	−2.2740	−.8523	−.2856	−.0588
Fy	This study	5	−.5845	−.1856	+.0370	+.0509	+.0178
K	This study	4	−.5181	−.2712	−.0834	−.0213	−.0032
P	This study	4	+.5147	+.4198	+.2498	+.1162	+.0300

[1] Based on 17 families.
[2] Based on 14 families.

Similar conclusions may be drawn from the data derived when phenylketonuria is treated as a recessive, except that the amount of information for each locus is less. The latter observation indicates that few if any errors were made in classifying heterozygotes on the basis of the stress test and the derived "cut-off" points.

It is perhaps worth noting that the scores for the P locus are suggestive of linkage. None of the scores are significant, but only 5 families were available for analysis with phenylketonuria treated as an intermediate dominant and 4 families with phenylketonuria treated as a recessive.

It is apparent that many more data are required to exclude loose linkage (.3 or .4). It is also apparent that such data can best be obtained by treating phenylketonuria as an intermediate dominant. Unfortunately, the available tests for detecting the heterozygote are relatively inefficient; hence, until they are improved, it would not seem desirable to pursue the linkage study.

SUMMARY

Linkage between phenylketonuria and the ABO, MNS, Rh, P, K, and Fy loci was examined by Morton's sequential method, treating phenylketonuria as an intermediate dominant and as a recessive.

Linkage with ABO, Rh, or MNS as close as .1 or less is excluded.

Linkage with K of .05 or less is excluded.

The data are insufficient to permit other conclusions.

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TABLE A. FAMILY DATA

In each family the father is listed first, followed by the mother and their children.

Fam.	No. in Ped.	Sex	Year of Birth	Sum of 1 and 2 hour phenyl-alanine levels in mgms %	Genotype	A	B	M	N	S	C	D	E	c	e	Fy[a]	K	P
1-P6	I-1	M	12	34.7	Phph	+	−	+	−	−	−	−	−	+	n	+	−	−
	2	F	11	38.0	Phph	−	−	+	−	+	+	+	−	+	n	+	+	−
	II-1	M	38	25.2	Ph?	+	−	+	−	+	−	+	−	+	n	+	+	−
	2	F	41	—	phph	+	−	+	−	+	+	+	−	+	n	+	+	−
	3	F	43	—	phph	−	−	+	−	+	−	+	−	+	n	+	−	−
2-P7	I-1	M	30	41.3	Phph	−	−	+	+	+	+	+	−	+	n	+	−	+
	2	F	29	39.8	Phph	+	−	+	+	−	−	−	−	+	n	+	−	+
	II-1	M	52	—	phph	+	−	+	+	+	+	+	−	+	n	+	−	+
	2	F	54	n	Ph?	+	−	+	+	−	−	−	−	+	n	+	−	+
	3	F	55	—	phph	−	−	−	+	−	−	−	−	+	n	−	−	+
	4	M	57	n	Ph?	−	−	+	+	−	−	−	−	+	n	+	−	−
3-P8	I-1	M	16	30.9	Phph	−	−	+	+	−	+	+	−	+	n	−	−	+
	2	F	20	39.9	Phph	−	−	+	+	−	+	+	−	+	n	+	−	+
	II-1	F	41	31.0	Phph	−	−	+	+	−	−	−	−	+	n	+	−	+
	2	M	42	—	phph	−	−	+	+	n	−	−	−	+	n	−	−	+
	5	M	54	—	phph	−	−	+	+	−	+	+	−	+	n	−	−	+
4-P9	I-1	M	16	31.6	Phph	−	+	+	+	−	−	+	+	+	+	+	−	−
	2	F	26	36.9	Phph	−	−	+	−	+	−	+	+	+	+	−	−	+
	II-3	M	48	18.8	Ph?	−	−	+	−	−	−	−	−	+	n	−	−	+
	4	M	54	—	phph	−	+	+	+	+	−	−	−	+	n	+	−	−
5-P13	I-1	M	24	20.6	Phph	−	−	+	+	+	+	+	−	+	n	−	−	−
	2	F	22	17.6	Phph	−	+	+	+	+	+	+	−	+	n	+	−	−
	II-1	M	T48	18.8	Ph?	−	+	+	+	+	+	+	−	+	n	+	−	−
	2	F	48	6.0	PhPh	−	+	+	−	+	+	+	−	−	n	+	−	−
	3	F	50	—	phph	−	+	+	+	−	+	+	−	+	n	+	n	−
6-P1	II-5	M	27	38.6	Phph	−	+	+	−	+	+	+	−	+	n	+	−	−
	6	F	28	30.0	Phph	−	−	+	+	+	−	−	−	+	n	−	−	−
	III-5	M	49	—	phph	−	+	+	+	+	+	+	−	+	n	+	−	−
	6	M	55	—	phph	−	−	+	−	+	+	+	−	+	n	−	−	−
7-P3	I-1	M	80	30.6	Phph	−	−	+	−	n	+	+	−	−	n	+	−	−
	2	F	82	19.1	PhPh	+	−	+	−	n	+	+	−	−	n	+	−	+
	II-1	M	12	13.2	PhPh	−	−	+	−	n	+	+	−	−	n	+	−	−
	2	M	14	28.9	Phph	+	−	+	−	−	+	+	−	−	n	+	−	−
	3	F	16	15.4	PhPh	−	−	+	−	n	+	+	−	−	n	+	−	+
	4	M	19	13.2	PhPh	+	−	+	−	n	+	+	−	−	n	+	−	+
8-P3	II-2[a]	M	14	28.9	Phph	+	−	+	−	−	+	+	−	−	n	+	−	−
	2[a]	F	17	33.7	Phph	−	−	+	+	+	+	+	−	+	n	+	+	+
	III-1	M	44	13.7	PhPh	−	−	+	+	−	+	+	−	−	n	+	+	−
	2	M	48	—	phph	+	−	+	+	−	+	+	−	−	n	+	n	−
	3	M	53	n	Ph?	+	−	+	−	+	+	+	−	+	n	+	−	+
	4	F	55	—	phph	+	−	+	+	−	+	+	−	−	n	+	−	−

TABLE A.—*Continued*

Fam.	No. in Ped.	Sex	Year of Birth	Sum of 1 and 2 hour phenylalanine levels in mgms %	Genotype	A	B	M	N	S	C	D	E	c	e	Fy[a]	K	P
9-P5	I-1	M	06	30.1	Phph	−	+	+	+	+	+	+	−	−	n	+	−	−
	2	F	16	17.6	Phph	+	−	+	+	+	+	+	+	+	+	+	−	−
	II-1	F	43	24.2	Ph?	+	+	−	+	+	+	+	−	−	n	+	−	−
	2	M	46	—	phph	+	−	+	−	−	+	+	+	+	+	+	−	−
10-P11	I-1	M	24	40.2	Phph	−	−	+	−	+	+	+	−	−	n	−	−	+
	2	F	29	29.8	Phph	+	+	−	+	+	+	+	−	−	n	+	+[b]	+
	II-1	F	—	—	phph	n	n	n	n	n	n	n	n	n	n	n	n	n
	2	F	50	31.2	Phph	+	−	+	+	+	+	+	−	−	n	−	−	+
	3	M	52	20.8	Ph?	+	−	+	+	+	+	+	−	−	n	n	−	+
11-P10	I-1	M	12	34.3	Phph	+	−	+	+	−	+	+	−	+	n	+	−	+
	2	F	12	n	Phph	+	+	−	+	n	+	+	−	+	n	+	−	+
	II-2	M	49	15.5	PhPh	+	−	+	+	n	+	+	−	+	n	+	−	+
	3	M	51	—	phph	+	+	−	+	−	−	−	−	+	n	+	−	+
12-P2	I-1	M	79	39.2	Phph	+	−	+	−	n	+	+	−	−	n	+	−	+
	2	F	78	15.8	PhPh	−	−	+	+	n	+	+	−	+	n	−	+	+
	II-1	F	08	23.7	Ph?	+	−	+	+	n	+	+	−	+	n	−	−	+
	2	M	10	31.5	Phph	−	−	+	+	n	+	+	−	+	n	−	+	+
	3	M	13	29.7	Phph	−	−	+	−	+	+	+	−	−	n	−	+	−
	4	M	15	26.0	Ph?	−	−	+	−	n	+	+	−	−	n	−	−	+
	5	F	22	3.9	PhPh	−	−	+	+	n	+	+	−	+	n	−	+	n
	6	F	28	13.3	PhPh	−	−	+	−	n	+	+	−	−	n	+	−	+
13-P2	II-2	M[c]	10	31.5	Phph	−	−	+	+	n	+	+	−	+	n	−	+	+
	2a	F	—	n	PhPh[d]	−	−	+	−	+	+	+	−	+	n	+	−	−
	III-1	M	41	15.4	PhPh	−	−	+	−	+	−	−	−	+	n	−	+	−
	2	M	44	16.2	PhPh	−	−	+	−	+	−	−	−	+	n	+	+	−
14-P2	II-3	M[c]	13	29.7	Phph	−	−	+	−	+	+	+	−	−	n	−	+	−
	3a	F	15	28.9	Phph	−	−	−	+	−	+	+	−	+	n	−	−	−
	III-3	F	41	17.8	PhPh	−	−	+	+	+	+	+	−	−	n	−	+	−
	4	F	44	28.3	Phph	−	−	+	+	−	+	+	−	−	n	−	−	−
	5	F	46	—	phph	−	−	+	+	+	+	+	−	−	n	−	+	−
	6	F	52	—	phph	−	−	+	+	−	+	+	−	−	n	−	+	−
15-P2	II-4	M[c]	15	26.0	Ph?	−	−	+	−	n	+	+	−	−	n	−	−	+
	4a	F	—	n	PhPh[d]	−	−	+	−	+	+	+	−	−	n	+	−	−
	III-3	M	42	15.7	PhPh	−	−	+	−	+	+	+	−	−	n	−	−	+
	4	F	46	10.7	PhPh	−	−	+	−	−	+	+	−	−	n	+	−	−
16-P14	I-1	M	18	24.3	Phph	−	−	+	+	n	+	+	−	+	n	+	−	−
	2	F	20	18.2	Phph	+	−	+	+	n	−	−	+	+	+	+	−	−
	II-1	M	40	23.7	Ph?	−	−	+	+	n	+	+	−	+	n	+	−	−
	2	M	44	11.7	PhPh	+	−	+	−	n	+	+	−	+	n	+	−	−
	3	M	52	—	phph	+	−	+	−	n	+	+	+	+	+	+	−	−

TABLE A.—*Continued*

Fam.	No. in Ped.	Sex	Year of Birth	Sum of 1 and 2 hour phenylalanine levels in mgms %	Genotype	A	B	M	N	S	C	D	E	c	e	Fy^a	K	P
17-P17	I-1	M	17	25.7	Phph	−	−	+	+	+	+	+	−	+	n	+	−	−
	2	F	20	20.7	Phph	−	+	+	−	+	+	+	−	+	n	+	−	−
	II-1	M	42	15.0	PhPh	−	+	+	+	+	+	+	−	+	n	+	−	−
	2	M	44	—	phph	−	+	+	−	+	+	+	−	−	n	+	n	−
18-P16	I-1	M	91	38.3	Phph	+	−	+	+	+	−	−	−	+	n	+	−	−
	2	F	96	52.8	Phph	−	−	+	+	−	−	−	−	+	n	+	−	−
	II-1	M	19	—	phph	+	−	+	+	−	−	−	−	+	n	n	−	−
	3	M	23	17.8	PhPh	−	−	+	+	−	−	−	−	+	n	+	−	−
	4	F	28	22.1	Ph?	+	−	+	−	+	−	−	−	+	n	+	−	−
	5	F	31	—	phph	+	−	+	+	+	−	−	−	+	n	n	−	−
20a-P18	I-3	M	07	44.5	Phph	−	−	+	−	+	+	+	−	+	n	−	−	−
	4	F	12	28.7	PhPh	+	−	−	+	+	+	+	+	+	+	+	−	+
	II-3	M	30	22.0	Phph	+	−	+	+	+	+	+	+	+	+	+	−	+
	5	F	51	24.6	Ph?	−	−	+	+	+	+	+	+	+	+	+	−	+
20b-P18	I-1	M	00	25.4	PhPh	+	−	−	+	−	−	−	−	+	n	+	−	+
	2	F	04	26.6	Phph	−	−	+	−	−	−	−	−	+	n	+	+	+
	II-1	F	29	19.9	Phph	+	−	+	+	−	−	−	−	+	n	+	−	+
	2	F	34	33.9	Phph	−	−	+	+	−	−	−	−	+	n	+	+	+
20c-P18	II-3	M[e]	30	22.0	Phph	+	−	+	+	+	+	+	+	+	+	+	−	+
	1	F[f]	29	19.9	Phph	+	−	+	+	−	−	−	−	+	n	+	−	+
	III-1	F	53	15.1	PhPh	+	−	−	+	+	+	+	−	+	n	+	−	+
	2	F	56	—	phph	+	−	+	+	−	+	+	−	+	n	+	−	+
21-P19	II-1	M	20	27.7	Phph	+	+	+	+	+	+	+	−	+	n	+	−	−
	2	F	24	16.0	Phph	+	−	+	+	−	+	+	−	−	n	+	−	+
	III-1	M	48	2.0	PhPh	+	−	+	+	−	+	+	−	−	n	+	−	+
	2	M	—	—	phph	+	−	−	+	−	+	+	−	−	n	+	−	−
21a-P19	I-1	M	88	26.7	Phph	−	−	+	−	−	+	+	−	−	n	+	−	+
	2	F	98	16.1	PhPh	+	−	−	+	−	+	+	−	+	n	−	−	+
	II-2	F[g]	24	16.0	Phph	+	−	+	+	−	+	+	−	−	n	+	−	+
	3	F	27	6.5	PhPh	+	−	+	+	−	+	+	−	−	n	+	−	+
22	I-1	M	28		Phph	−	+	+	+	+	+	+	+	+	+	−	−	+
	2	F	28		Phph	−	+	−	+	−	+	+	−	+	n	+	−	+
	II-2	F	51		Phph	−	−	+	+	+	−	+	+	+	+	+	−	+
	4	F	56		phph	−	+	−	+	−	+	+	−	+	n	−	−	+
26-P21	I-1	M	30	38.9	Phph	−	−	+	+	+	−	−	−	+	n	+	−	−
	2	F	35	19.9	Phph	+	−	−	+	−	+	+	−	+	n	+	−	−
	II-1	M	57	—	phph	+	−	−	+	−	+	+	−	+	n	+	−	−
	2	M	57	—	phph	+	−	+	+	+	+	+	−	+	n	+	−	−

TABLE A.—*Continued*

Fam.	No. in Ped.	Sex	Year of Birth	Sum of 1 and 2 hour phenyl-alanine levels in mgms %	Genotype	A	B	M	N	S	C	D	E	c	e	Fy[a]	K	P
27-P22	I-1	M	26	48.9	Phph	+	−	+	+	+	−	+	+	+	−	+	−	+
	2	F	31	55.7	Phph	−	−	+	−	+	+	+	−	+	n	+	−	+
	II-2	M	50	30.0	Phph	−	−	+	+	+	−	+	+	+	+	+	−	+
	3	M	52	—	phph	+	−	+	+	n	−	+	+	+	+	+	−	+
	4	F	53	51.0	Phph	−	−	+	−	+	−	+	+	+	+	+	−	+
	5	M	55	22.0	Ph?	+	−	+	−	+	−	+	+	+	+	+	−	+
	6	F	56	—	phph	+	−	+	−	+	+	+	+	+	+	+	−	+
	7	M	57	40.4	Phph	−	−	+	+	+	+	+	+	+	+	+	−	+
	8	M	58	—	phph	−	−	+	+	−	−	+	+	+	+	+	−	+

[a] His parents are I-1 and I-2 of 7-P3.
[b] Tested with k.
[c] His parents are I-1 and I-2 of 12-P2.
[d] Assumed.
[e] His parents are I-3 and I-4 of 20a-P18.
[f] Her parents are I-1 and I-2 of 20b-P18.
[g] Mother of Family 21-P19.

Phenylketonuria: A Linkage Study Using Phenylalanine Tolerance Tests

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INTRODUCTION

THE DATA of Munro (1947) were compatible with loose linkage between the phenylketonuria locus and the ABO blood group locus. When they were reanalyzed by Penrose (1951b) with some additional families, they still did not show a convincing deviation from a hypothesis of no linkage. However, the introduction by Hsia, Driscoll, Troll and Knox (1956) of the phenylalanine tolerance test to detect heterozygotes suggested a more powerful method of adding to the available linkage data.

OUTLINE OF INVESTIGATION

It was therefore decided that available relatives of phenylketonurics should be tested for heterozygosity at the phenylketonuric locus and also blood-grouped.

To establish our own criteria for detecting the heterozygotes, tolerance tests were carried out on two known classes of persons as indicated in table 1. A group of parents of phenylketonurics constituted our heterozygous class. The normal class consisted mainly of University personnel and their spouses. This group might conceivably contain a heterozygote, but the frequency of heterozygotes in such a population of persons not known to have a phenylketonuric relative is likely to be no greater than 1 in 80.

TOLERANCE TEST

An accurate dose of 1.0 G. per kilogram body weight of *l*-phenylalanine was dissolved in boiling water and diluted with an equal volume of cool pineapple juice with added sugar, so that the final solution (about 300 ml.) contained approximately 2.5 G/100 ml. This was administered by mouth after an overnight fast.

To ascertain the best method of discriminating between a heterozygote and a homozygous normal, 38 persons (Series I) belonging to one or the other group were tested at 1 hour, 2 hours and 4 hours in addition to a fasting sample. Subsequently the data on the 4-hour levels were extended by the testing of an extra

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TABLE 1. NUMBERS OF PERSONS TESTED IN THE KNOWN CLASSES

		Phenylalanine estimations				Tyrosine estimations			
		Hours after phenylalanine dose							
		0	1	2	4	0	1	2	4
Series I	Presumed heterozygotes (parents)	18	19	18	18	4	4	4	12
	Presumed homozygous normals	20	20	20	20	7	7	7	28
Series II	Presumed heterozygotes (parents)	—	—	—	21	—	—	—	21
	Presumed homozygous normals	—	—	—	25	—	—	—	25
Total		38	39	38	84	11	11	11	86

21 heterozygotes and 25 normal controls (Series II). "Unknowns" were studied only after the 4-hour interval.

Method of estimating l-phenylalanine. The *l*-phenylalanine content of the plasma was assayed by the decarboxylase method of Udenfriend and Cooper (1953) as modified by Knox and Messinger (1958).

Some idea of the repeatability of the estimates can be gained by considering the standard deviation of the duplicates, which is approximately 4μM/l. for each 100μM/l. This is augmented by error in the standard line, and by variations between experiments done on different days. There are additional sources of variation in the stage of deproteinization, and in the physiological response of the same individual to repeated tests. The following are the observed results in chronological order from repeat tolerance tests on each of 4 subjects:

584, 528μM/l. — at 1 hour, subject 14 Appendix Ia
830, 900μM/l. — at 1 hour, subject 13 Appendix Ia
1018, 1009μM/l. — at 1 hour, subject 2 Appendix Ia
291, 270μM/l. — at 4 hours, subject 89. I. 2 Appendix Ic

These 4 pairs give 5μM/l. (for every 100μM/l.) as an estimated standard deviation for the calculated phenylalanine concentration in the plasma.

The results of the phenylalanine estimations for the known series (Fig. 1) are given in Appendix I and for the "unknown" individuals (Fig. 2) in columns 6, 7 of Appendix II.

Tyrosine estimations

The concentration of *l*-tyrosine was measured in the 4-hour samples in heterozygotes and normal homozygotes of Series I by the method of Udenfriend and Cooper (1952). The results are given as part of Appendix I.

Regression of phenylalanine levels on weight of subject

In the normals of Series I a suggestion of a correlation between weight and phenylalanine levels had been noted in the 1, 2 and 4-hour samples, and several heavy individuals were therefore included in the normals of Series II to extend

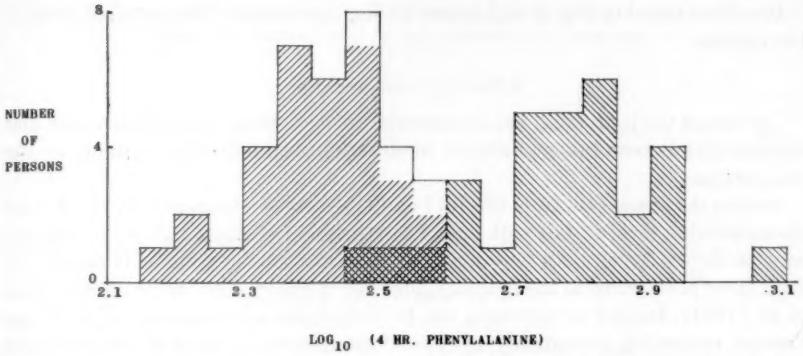

FIG. 1. Histogram showing the distribution of plasma phenylalanine levels at 4 hours in assumed normal homozygotes, ▨, and in known heterozygotes, ▧. The encompassing unbroken line indicates the combinet distribution.

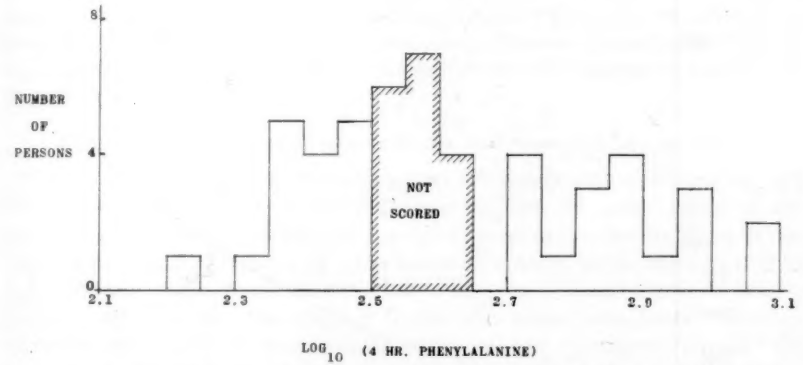

FIG. 2. Histogram showing observed distribution of 4-hour phenylalanine levels in the group of "unknowns," (relatives of phenylketonurics).

the weight range. A convincing relationship was thus shown (see Fig. 3 for scatter diagram). The regression curve is defined by

$$y = 2.559 - 0.0602x + 0.0059x^2$$

(where $y = \log_{10}$ (phenylalanine level at 4 hr). and $x =$ body weight in 10 Kg. or dose in G.). It is perhaps justifiable to assume that the apparent lack of regression among the heterozygotes is due to the narrower weight range. Physiologically, a relationship between weight and phenylalanine level would be expected in *both* groups when a challenge dose is given on the basis of body weight rather than of surface area.

It will be noted in Fig. 3 that below 80 Kg. the weight effect is small even in the normals.

Effects of sex and age

The sex of the individual has apparently little effect on the tolerance test and no account has been taken of the sex in classifying an individual into one of the two groups.

Among the normal controls (Series I and II) there is a tendency for the 4-hour phenylalanine level to fall with age. The regression is slight but is just significant at the 5% level, both before and after correction for weight. However, no such effect is observed in the heterozygotes nor in either class in the data of Hsia et al. (1957). Insofar as anything can be judged from the present series of unknowns, consisting, presumably, of approximately equal mixture of heterozygotes

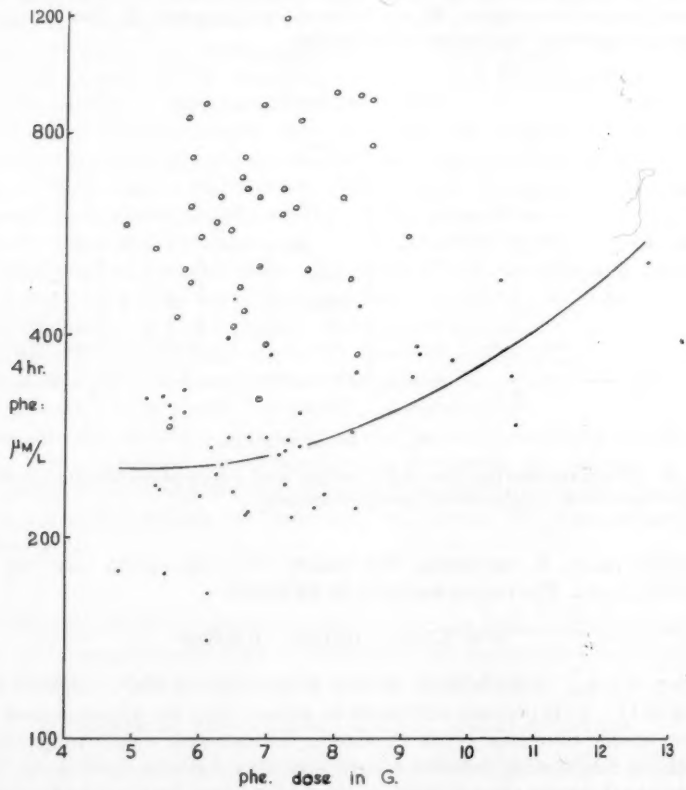

FIG. 3. Effect of dose (or body weight) on 4-hour plasma phenylalanine level. A parabola, $y = 2.559 - 0.0602x + 0.0059x^2$, was fitted to the points representing homozygous normals (solid circles). The heterozygotes are represented by unfilled circles.

TABLE 2. ESTIMATES, $D/\bar{S}$, OF THE POWERS OF VARIOUS METHODS OF DISCRIMINATING BETWEEN HETEROZYGOTES AND HOMOZYGOUS NORMALS

Measurement	Power, $D/\bar{S}$		
	Present series	Hsia et al. (1956, 1957)	Knox & Messinger (1958)
Plasma phenylalanine, (phe.) 0 hour, a,	1.8	1.2	1.9
1 hour, b,	1.2	3.2	—
2 hour, c,	2.4	2.7	—
4 hour, d,	2.7	2.5	—
Area = $\frac{1}{2}(a + 2b + 3c + 2d)$	3.2	—	—
Log_{10}(4hr. phe.) corrected for weight.	3.3	—	—
Log_{10}(4hr. phe.) for persons < 80Kg.	3.1	—	—
Log_{10}(phe. at 1hr./tyrosine at 1hr.)	—	2.0	—

and normal homozygotes, the age effect if present seems to be in the opposite direction. No age correction has therefore been made on these data, but children under 17 have been avoided in case phenylalanine metabolism is different in them. It is of course impossible to obtain a group of known heterozygous children, for a direct testing of this, since the phenylketonuric homozygotes hardly ever bear children.

Discriminating between heterozygotes and normal homozygotes

The discriminating power of a measurement is best described in terms of $D/\bar{S}$ (Penrose 1951a) where D is the difference between the mean values of the measurement in the two groups and $\bar{S}$ is the average of the two standard deviations. Some values of $D/\bar{S}$ are given in table 2. Contrary to the findings of Hsia and Steinberg (1960), it will be seen that in the present data the 4-hour level is the best single sample, and in Series II only this sample was tested. The concurrent testing of the 4-hour tyrosine level does not apparently improve the discrimination, but there are indications that, in spite of the marked correlations among the 0, 1, 2 and 4-hour phenylalanine levels, a more time-consuming criterion based on all four of these phenylalanine levels would have been slightly more powerful.

The unknowns tested, with one exception, proved to be under 80 Kg., the weight below which the regression with weight appeared small. The one result requiring weight correction was that on 57.II.6. In view of these light weights in the "unknown" group, the discriminating criterion was based only on the *uncorrected* 4-hour levels of those members of the "known" groups who weighed less than 80 Kg. i.e. 31 heterozygotes and 33 normal homozygotes. In classifying the unknowns, it would be reasonable to discard all those individuals for whom the odds that they belonged to a particular genotype were less than 4 to 1; in other words, those for whom 4AB > AC or 4EF > EG (see Fig. 4). Those observations with odds lying *between* 4:1 and 50:1 could be included in the linkage calculations when appropriate weightings for the two alternative genotypes were

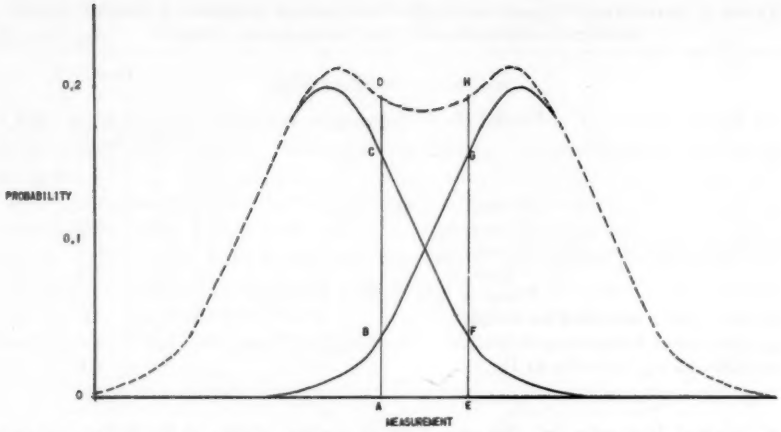

FIG. 4. Diagram to indicate the parts of two hypothetical normal distributions which fall in the "overlap zone", ADHE, in which an individual cannot be assigned with at least 80% confidence, to a particular one of the two populations represented in these distribution curves. In the "overlap zone" 4AB > AC and 4EF > EG (see text). In this diagram, $D/\bar{S} = 2\frac{1}{2}$ and the combined distribution (– – –) is bimodal.

utilized. The decisions on classification based on these arguments are given in Appendix II, column 8, together with the probability weights where necessary.

MATERIAL FOR LINKAGE STUDY

The 43 propositi were almost exclusively ascertained through their residence in mental deficiency hospitals, particularly those in the London and Home Counties areas of England.

Diagnosis of each propositus was based on the presence of phenylpyruvic acid in the fresh urine by the ferric chloride test and sometimes also on the fasting blood phenylalanine level which was determined approximately in 15 of these patients.

In each family, the blood groups, Gm(a) type and haptoglobin types were tested on the normal and abnormal individuals as listed in Appendix II. Many individuals (indicated (N) in column 8 Appendix II) have not been specifically tested for phenylketonuria, but in view of their apparently normal intelligence there is every reason to believe that very few, if any, affected individuals were overlooked. Tolerance tests were not performed on the sibs, because they would usually have provided less additional information than similar tests performed on other relatives.

It was decided to extend those 17 pedigrees (some are shown in Fig. 5–11) in which, knowledge of the carrier status of a grandparent immediately increased the information relating to a possible ABO:phenylketonuria linkage. Thus, if a parent was known to be homozygous at the ABO locus no extension was usually undertaken, whereas if a parent was of group A_1B, for example, an attempt

Key to pedigrees. (Figs. 5–11)

■ Phenylketonuric.
□ Not phenylketonuric.
⊠ Mentally deficient, not definitely known to be phenylketonuric.
↖ Propositus.
Fr Dizygotic twins.
? Twins, zygosity uncertain.
Stillbirth.
• Miscarriage.
△ Sex unspecified.

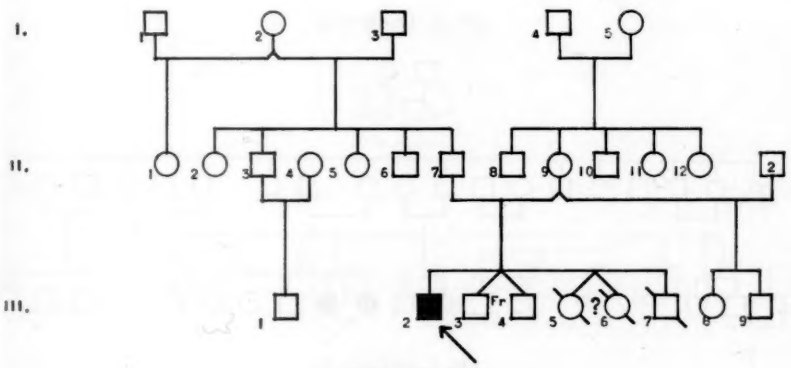

FIG. 5. Pedigree 57

was made to do tolerance and blood group testing on both grandparents and on the other children of these grandparents (i.e., aunts and uncles of the propositus). A few cousins were also tested if they had one parent who was found to be heterozygous at both the ABO and phenylketonuria loci. Pedigress 56 and 57 of Penrose (1951b) are among those extended (56, 57).

The choice of subjects on whom tolerance tests were performed ensured that the *a priori* odds that a tested individual was a heterozygote or a homozygous normal were even, if one ignores the unlikely eventuality of more than two grandparents of a phenylketonuric carrying the gene.

RESULTS OF LINKAGE CALCULATIONS

The probability ratio (or backward odds $\lambda\theta$) is defined as the probability of occurrence of the family data if the frequency of recombination is really some value, θ, compared with the probability of this occurrence if the recombination

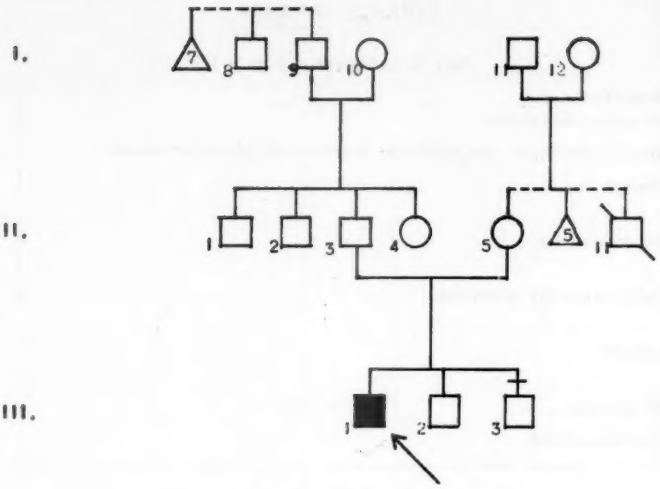

FIG. 6. Pedigree 62

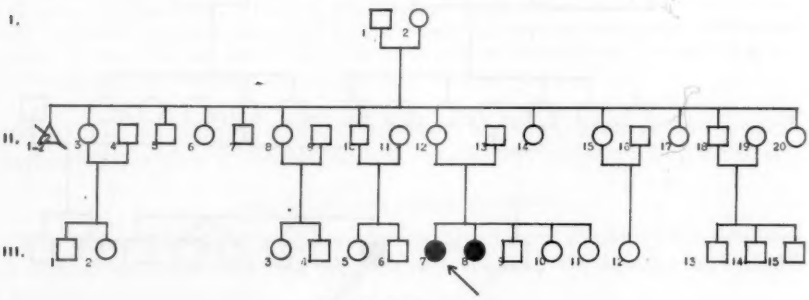

FIG. 7. Pedigree 63

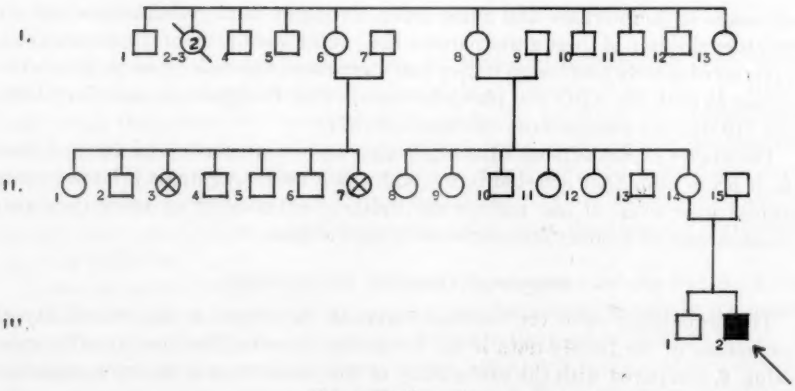

FIG. 8. Pedigree 64

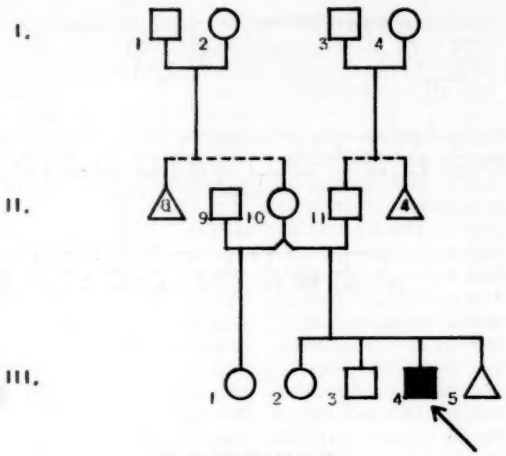

FIG. 9. Pedigree 66

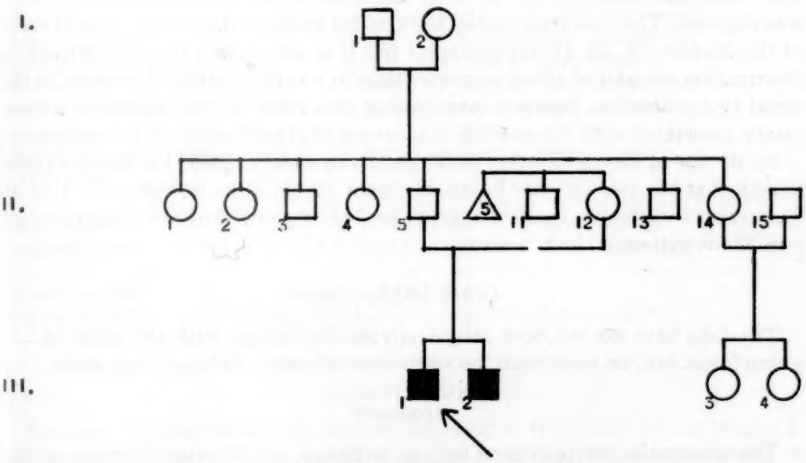

FIG. 10. Pedigree 73

frequency is really 0.5. The $\log_{10}$ of this ratio is the lod score (Smith 1953) called the z score by Morton (1955). The lod scores for four values of θ are given in table 3 for the ABO and phenylketonuria loci for 17 extended pedigrees and for the 15 simple families which gave information for this linkage test. They are compared with similar lod scores (z) of Hsia and Steinberg (1960) and also with those calculated from the data of Munro (1947) and Penrose (1951b). The computations were abbreviated by the use of Morton's (1955 and 1957) tabulations, and by the use of the electronic computer program of Simpson (1958). There

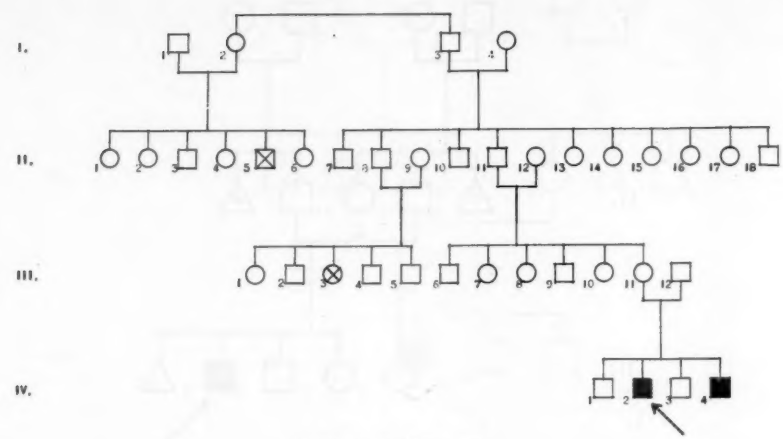

FIG. 11. Pedigree 75

was "complete selection" at the ABO locus, hence no ascertainment correction was required. The tolerance studies have added considerably to the data in some of the families (4, 13, 17, for example) but it is not certain that the reward in information per unit of effort is greater than in a study restricted entirely to the usual two generation families. Any gain is also offset by the increased uncertainty associated with the possible inaccuracy of classification of heterozygotes.

By the use of Bayes' theorem as advocated by Smith (1959) the present probability that the two loci are linked is shown (table 3) to be still only 1 in 10 (assuming with Smith, that the *a priori* probability of linkage is approximately 1 in 22 for autosomal loci in man).

Other linkage tests

The data have not yet been fully analyzed for linkage with any other blood-group locus, but, on inspection, no suggestion of such a linkage is apparent.

SUMMARY

The phenylalanine tolerance test as a means of detecting carriers of the phenylketonuria gene has been shown to be improved by correcting for the dose given. The four-hour plasma sample is better than the fasting sample or the one- or two-hour samples for this discrimination which is apparently not improved by estimating the plasma tyrosine level concurrently.

The four-hour test has been used in tracing the gene through three generations of several families as part of a linkage study. Data relating to the ABO and phenylketonuria pair of loci are similar to those of Munro (1947) and Penrose (1951b) in deviating in the direction of linkage, but even when all the available data (from 74 informative families) are combined, the evidence for linkage is weak.

TABLE 3. LOD (z) SCORES RELATING TO THE ABO:PHENYLKETONURIA PAIR OF LOCI

Pedigree	Lod (z) scores $\theta = 0.1$	$\theta = 0.2$	$\theta = 0.3$	$\theta = 0.4$	
56	−0.3650	−0.2043	−0.0897	−0.0219	
57	−0.5509	−0.2554	−0.1113	−0.0346	
61	−0.0304	−0.0202	−0.0090	−0.0021	
62	+0.0125	+0.0165	+0.0158	+0.0104	
63	+0.6130	+0.5704	+0.4064	+0.2043	
64	−0.1426	−0.0883	−0.0412	−0.0105	
65	−0.4437	−0.2518	−0.1347	−0.0555	
66	−0.1404	−0.1324	−0.0965	−0.0491	
67	+0.0087	+0.0049	+0.0022	+0.0006	
68	−0.2348	−0.0626	−0.0042	+0.0043	
69	−0.3767	−0.1548	−0.0580	−0.0132	
70	+0.1565	+0.0994	+0.0462	+0.0121	
71	+0.2553	+0.2041	+0.1461	+0.0792	
72	−0.3597	−0.1447	−0.0535	−0.0119	
73	+0.2632	+0.2546	+0.1638	+0.0635	
74	+0.0747	+0.0392	+0.0164	+0.0039	
75	+0.9041	+0.6774	+0.4417	+0.1904	
Total (17 peds.)	−0.3562	+0.5520	+0.6405	+0.3789	
Small pedigrees (15)	−0.0644	+0.0305	+0.0265	+0.0088	
Munro (1947) (17 peds.)	−1.8407	−0.1316	+0.2673	+0.1439	
Penrose (1951b) except 56 & 57 (11 peds.)	−0.7859	−0.1226	+0.0253	+0.0178	
Hsia & Steinberg (1960) table 2. (14 peds.)	−1.0060	−0.3065	−0.0803	−0.0128	
Total scores (z)	−4.0532	+0.0218	+0.8793	+0.5366	
Probability ratio (antilog of z)	8.8×10^{-5}	1.05	7.57	3.44	1 (at $\theta = 0.5$)

Average probability ratio, A, (when $\theta < 0.5$) = 2.28
Estimated probability of linkage = A/(A + 21) (from (7) of Smith, 1959.)
= 0.10

ACKNOWLEDGMENT

Families of phenylketonuria were contributed to this study by Professors L. S. Penrose, C. E. Dent, J. R. Squire and Drs. B. H. Kirman, B. W. Richards, J. D. Blainey, A. Shapiro, D. H. Thomas and D. Murphy. Their assistance in this way has been much appreciated.

We offer our thanks also to those who volunteered for the various tests involved, and to Miss Ruth Marshall, Miss Pat Giffon and Miss Lesley Anderson, who were responsible for much of the skilled execution of these tests in the laboratory.

Blood-grouping sera were supplied through the kindness of Drs. A. E. Mourant, D. Lehane and P. L. Mollison.

The computer programme was run by H. R. Simpson at Rothamsted on the Elliott 401, made available to us through the courtesy of Dr. F. Yates.

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Appendix Ia. Results of tolerance tests

Series I. Normal homozygotes

No.	Sex	Age	Dose 0.1 G/Kg.	Plasma phenylalanine level in μM/l.					Plasma tyrosine level in μM/l.				Phe/tyr at 4 hr.
				Fasting a	1 hr b	2 hr. c	4 hr. d	Area ½ (a + 2b + 3c + 2d)	Fasting	1 hr.	2 hr.	4 hr.	
1	M	31	7.31	64	1069	580	280	2251	—	—	—	—	—
2	F	25	5.77	52	1009	679	332	2386	—	—	—	—	—
3	F	16	5.34	65	808	497	244	1830	—	—	—	—	—
4	M	42	9.76	95	1421	880	368	3156	—	—	—	—	—
5	F	23	5.77	56	848	709	306	2246	—	—	—	—	—
6	M	27	7.17	60	987	600	266	2183	—	—	—	—	—
7	F	39	6.24	84	984	498	249	2022	—	—	—	—	—
8	F	24	5.45	83	888	582	324	2127	—	—	—	—	—
9	M	27	8.33	99	840	680	351	2260	—	—	—	—	—
10	F	34	5.56	97	1020	765	300	2516	—	—	—	—	—
11	M	29	7.26	68	756	525	270	1847	—	—	—	—	—
12	M	51	8.33	72	576	598	222	1731	—	—	—	—	—
13	F	33	4.81	52	900	492	178	1842	55	149	200	228	0.78
14	F	35	6.13	47	528	378	141	1260	101	127	191	209	0.67
15	M	33	6.70	67	792	516	219	1818	104	131	151	151	1.45
16	F	37	6.13	64	636	495	165	1575	68	162	135	172	0.96
17	M	26	7.36	77	792	408	215	1658	103	141	145	174	1.23
18	M	41	10.49	88	1140	876	483	2981	71	100	160	153	3.16
19	M	30	6.17	64	672	564	273	1823	81	113	136	149	1.83
20	M	34	6.99	54	465	468	282	1476	—	—	—	135	2.09
n		20	20	20	20	20	20	20	7	7	7	8	8
Mean		31.9	6.85	70.4	857	590	273	2049	83	132	160	171	1.52
SD		7.9	—	15.9	226	139	78	472	19.6	19.1	26.1	32.1	0.83

APPENDIX IB

Series I. Heterozygotes

No.	Sex	Age	Dose 0.1 G/Kg	Plasma Phenylalanine level in μM/l.					Plasma tyrosine level in μM/l.				Phe/tyr. at 4 hr.
				Fasting a	1 hr. b	2 hr. c	4 hr. d	Area ½ (a + 2b + 3c + 2d)	Fasting	1 hr	2 hr.	4 hr.	
98.I.2	F	41	6.06	77	940	—	—	—	—	—	—	—	—
98.I.1	M	44	8.31	82	994	691	372	2444	—	—	—	—	—
73.II.5	M	41	7.49	108	1195	1195	841	3882	—	—	—	—	—
73.II.12	F	35	5.63	—	1144	906	426	—	—	—	—	—	—
61.II.5	M	40	6.65	110	1296	1075	738	3702	—	—	—	—	—
61.II.7	F	37	8.35	118	1822	1222	914	4628	—	—	—	—	—
92.I.1	M	53	5.83	138	1044	774	480	2754	94	94	137	148	3.24
92.I.2	F	47	6.49	107	1440	915	411	3277	52	98	117	127	3.24
81.I.1	M	42	9.09	60	1152	1062	561	3336	144	161	179	195	2.88
81.I.2	F	42	6.27	91	1080	888	645	3102	126	122	163	186	3.47
91.I.2	F	32	5.86	110	1836	1290	738	4564	—	—	—	—	—
67.II.2	F	37	8.01	106	990	1206	924	3776	—	—	—	121	7.64
93.I.1	M	56	6.86	96	1128	1008	642	3330	—	—	—	107	6.00
93.I.2	F	45	7.22	102	1260	1302	1182	4446	—	—	—	67	17.64
84.I.2	F	70	6.67	113	1332	1146	663	3771	—	—	—	193	3.44
—	M	66	5.77	89	996	744	501	2657	—	—	—	104	4.82
63.II.13	M	32	6.61	93	432	684	690	2195	—	—	—	97	7.11
63.II.12	F	31	5.79	87	924	840	846	3073	—	—	—	94	9.00
79.I.1	M	43	7.58	109	1260	990	501	3301	—	—	—	119	4.21
n		10	19	18	19	18	18	17	4	4	4	12	12
Mean		44.1	6.87	99.8	1172	996	671	3926	104	119	149	130	6.06
SD		14.4	—	17.6	314	203	214	712	40.4	30.8	27.4	42.0	4.17

APPENDIX Ic

Series II

Normals							Heterozygotes						
No.	Sex	Age	Dose 0.1 G/Kg.	4 hr. phe. μM/l.	4 hr. tyr. μM/l.	Phe/tyr. at 4 hr.	Ped. No.	Sex	Age	Dose 0.1 G/Kg.	4 hr. phe. μM/l.	4 hr. tyr. μM/l.	Phe/tyr. at 4 hr.
21	F	29	6.42	282	128	2.20	79.I.2	F	39	8.22	486	148	3.28
22	M	25	7.04	375	124	3.02	56.II.4	M	53	6.86	321	111	2.89
23	M	25	8.26	287	126	2.28	56.II.3	F	48	6.22	591	103	5.74
24	F	64	5.47	177	119	1.49	62.II.5	F	36	6.45	576	170	3.39
25	M	40	10.71	293	120	2.44	63.II.3	M	36	6.63	432	161	2.68
26	F	23	5.20	321	161	1.99	76.I.2	F	63	7.20	606	128	4.73
27	M	34	7.47	273	164	1.66	77.I.1	M	56	6.58	471	99	4.76
28	M	23	7.26	240	105	2.29	77.I.2	F	52	8.54	900	128	7.03
29	F	29	6.31	258	167	1.54	—	M	56	7.40	621	135	4.60
30	M	59	7.49	306	179	1.71	—	M	57	5.99	561	157	3.57
31	M	37	9.26	375	231	1.62	—	F	52	8.54	768	223	3.44
32	F	36	6.49	234	126	1.86	57.II.7	M	40	6.97	387	114	3.39
33	F	25	5.54	315	161	1.96	57.II.9	F	36	5.83	624	167	3.74
34	M	39	12.67	513	188	2.73	—	F	52	6.92	888	148	6.00
35	F	21	5.99	231	174	1.33	—	F	53	6.06	888	175	4.80
36	F	34	8.35	441	241	1.83	—	F	47	8.10	645	146	4.42
37	M	32	7.70	222	168	1.32	89.I.1	M	42	6.86	507	160	3.17
38	M	37	10.65	348	244	1.42	89.I.2	F	36	5.54	291*	181	1.61
39	M	53	6.67	219	140	1.56	64.II.15	M	43	7.22	660	116	5.69
40	M	44	9.22	384	209	1.84	64.II.14	F	34	4.88	588	100	5.88
41	M	40	5.40	237	143	1.66	—	F	73	5.31	540	108	5.00
42	M	52	7.85	233	119	1.96							
43	M	23	6.40	396	192	2.06							
44	F	56	6.72	282	182	1.55							
45	M	32	9.17	348	124	2.81							
Series I + II excluding 98.I.2													
n		45	45	45	32	32			39	39	39	33	33
Mean		34.4	7.26	290.2	162.4	1.88			45.9	6.84	626	137.5	4.92
SD		10.6	1.117	79.2	37.8	0.491			10.8	1.014	193.5	35.9	2.80

* Second tolerance test gave value of 271 μM/l.

Appendix II: Linkage data

Key

Column

1. Pedigree number. Brackets indicate twins. * = Propositus.
2. Pedigree number of parents.
3. Sex.
4. Year of birth.
5. Living (l.) or dead (d.), with date of death when known.
6. Dose of *l*-phenylalanine in grammes, (0.1G. per Kg. body weight).
7. 4-hr. phe. = plasma phenylalanine level 4 hours after loading dose. Results italicized apply to known heterozygotes. For 64.II.7, the fasting phenylalanine level is indicated as F186.
8. Classification at phenylketonuria locus.
   - ph = phenylketonuria allele.
   - Ph = normal allele.
   - N = normal phenotype (not excreting phenylpyruvic acid); therefore Ph Ph or Ph ph genotype.
   - (N) = normal intelligence, therefore presumed Ph Ph or Ph ph genotype.
   - Ph Ph = homozygous normal on tolerance test.
   - (Ph Ph) = normal person at random, therefore assumed Ph Ph (probability 0.99).
   - Ph ph = heterozygote on basis of tolerance test or of phenylketonuric offspring.
   - (Ph ph) = grandparent of phenylketonuric; assumed heterozygote since spouse is not.
   - ph ph = proven phenylketonuric.
9. e = examined

Blood group systems

10. *ABO*
11. *MNS* Anti-sera used: anti-*M*, anti-*N*, anti-*S*. Genotypes have been inferred where possible.
12. Rhesus — Key to phenotypes — Most likely genotype

rr = D − C − C^w − c + E − e +	*cde/cde*
R_1r = D + C + C^w − c + E − e +	*CDe/cde*
R_1^wr = D + C + C^w + c + E − e +	*C^wDe/cde*
R_1R_1 = D + C + C^w − c − E − e +	*CDe/CDe*
$R_1^wR_1$ = D + C + C^w + c − E − e +	*C^wDe/CDe*
R_1R_2 = D + C + C^w − c + E + e +	*CDe/cDE*
R_2R_2 = D + C − C^w − c + E + e −	*cDE/cDE*
R_2r = D + C − C^w − c + E + e +	*cDE/cde*
$R''r$ = D − C − C^w − c + E + e +	*cdE/cde*
$R''r(D^u)$ = D ± C − C^w − c + E + e + (77.I.1 only)	*cD^uE/cde*
R_1R'' = D + C + C^w − c + E + e +	*CDe/cD^wE* (77.II.1) *CDe/cdE* (72.II.4)
R_2 = D + C − C^w − c + E + (93.I.2 only)	*cDE/cde* *cDE/cDE*
R_0r = D + C − C^w − c + E − e +	*cDe/cde*

13. P = P phenotype, P + or −: anti-serum used: anti-P_1.
14. Le(a) = Lewis red cell phenotype, Le(a+) or Le(a−).
15. Le(b) = Lewis red cell phenotype, Le(b+) or Le(b−).
16. K = Kell phenotype, K+ or −: anti-serum used: anti-*K*.
17. Lu(a) = Lutheran phenotype, Lu(a+) or Lu(a−).
18. Fy(a) = Duffy phenotype, Fy(a+) or Fy(a−).
19. Sec. = ABH secretion phenotype; + = secretor, − = non-secretor.
20. Gm(a) = Gm(a) phenotype, Gm(a+) or Gm(a−).
21. Hp = Haptoglobin phenotype, 1.1, 2.1, 2.2.

Questions about relevant consanguinity were asked in all families. No example was found, except that in family 84 (page 317).

APPENDIX II—*Continued*

1 Ped. no.	2 Parents	3 Sex	4 Year of birth	5 Living or dead	6 Dose G	7 4 hr. phe. μM/l.	8 Class.	9 e	10 *ABO*	11 *MNS*	12 *Rh*	13 P	14 Le(a)	15 Le(b)	16 K	17 Lu(a)	18 Fy(a)	19 Sec.	20 Gm(a)	21 Hp
							Family 56													
I 1	·	M	·	d aged 18	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
I 2	·	F	·	d aged 62	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
II 1	I 1, 2	M	1901	l	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
II 2	I 1, 2	F	1906	l	7.99	780	Ph ph	e	A_1	*MsNs*	R_1r	+	−	+	−	−	+	+	+	2.1
II 3	I 1, 2	F	1909	l	6.22	*591*	Ph ph	e	*B*	*MSMs*	R_1r	−	−	·	−	−	+	+	−	2.1
II 4	·	M	1904	l	6.86	*321*	Ph ph	e	*O*	*MsNs*	$R_1^wR_1$	+	−	+	−	−	−	+	−	2.2
III 1	II 3, 4	M	1933	l	·	·	(N)	e	*O*	*MsNs*	$R_1^wR_1$	+	−	·	−	−	·	·	·	·
III 2	II 3, 4	F	1935	l	·	·	(N)	e	*O*	*MsNs*	$R_1^wR_1$	+	−	·	−	−	·	·	·	·
III 3*	II 3, 4	M	1941	l	·	·	ph ph	e	*O*	*MsMs*	R_1^wr	+	−	+	−	−	+	·	−	2.2
III 4	II 3, 4	M	1943	l	·	·	(N)	e	*O*	*MsNs*	R_1r	+	−	·	−	−	·	+	·	·
III 5	II 3, 4	M	1943	l	·	·	(N)	e	*B*	*MSMs*	$R_1^wR_1$	+	−	·	−	−	·	·	·	·
							Family 57													
I 1	·	M	·	d	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
I 2	·	F	1870	d 1942	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
I 3	·	M	1866	d 1938	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
I 4	·	M	1892	d 1948	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
I 5	·	F	1897	l	6.72	351	N	e	A_1	*MsNs*	*rr*	+	+	−	−	−	+	−	−	2.1
II 1	I 1, 2	F	1894	l	·	·	(N)	e	A_2	·	·	·	·	·	·	·	·	+	·	·
II 2	I 2, 3	F	1904	l	7.26	312	.86 Ph Ph .14 Ph ph	e	*B*	*MsMs*	*rr*	+	−	·	−	−	+	+	+	2.2
II 3	I 2, 3	M	1905	l	6.76	514	N	e	*B*	*MsMs*	*rr*	+	+	·	−	−	+	−	+	1.1
II 4	·	F	·	l	·	·	(Ph Ph)	·	·	·	·	·	·	·	·	·	·	·	·	·
II 5	I 2, 3	F	1909	l	6.76	306	.89 Ph Ph .11 Ph ph	e	*O*	*MsMs*	R_1r	+	+	−	−	−	+	−	+	2.2

II 6	I 2, 3	M	1912	l	10.08	762†	Ph ph	e	*O*	*MsMs*	R_1r	+	−	+	−	−	−	+	+	·
II 7	I 2, 3	M	1918	l	6.97	*387*	Ph ph	e	*B*	*MSMs*	R_1r	+	+	·	−	−	−	−	+	2.2
II 8	I 4, 5	M	1920	l	7.67	387	N	e	A_1	*MsNs*	*rr*	+	−	+	−	−	+	+	·	2.1
II 9	I 4, 5	F	1922	l	5.83	*624*	Ph ph	e	A_1B	*NsNs*	*rr*	+	−	·	−	−	+	+	−	2.1
II 10	I 4, 5	M	1924	l	·	·	(N)	e	A_1B	·	·	+	·	·	·	·	·	·	·	·
II 11	I 4, 5	F	1926	l	5.36	279	Ph Ph	e	A_1	*NsNs*	*rr*	+	−	+	−	−	+	+	+	1.1
II 12	I 4, 5	F	·	d aged 2	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
II 13–14	·	M	·	·	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
III 1	II 3, 4	M	1930	l	6.76	381	N	e	A_1B	*M*	R_1r	+	−	·	−	−	+	+	·	2.1
III 2*	II 7, 9	M	1941	l	·	·	ph ph	e	A_1B	*MsNs*	R_1r	+	−	·	−	−	+	+	−	2.2
III 3	II 7, 9	M	1948	l	·	·	N	e	A_1	*MsNs*	*rr*	+	·	·	−	−	+	−	·	·
III 4	II 7, 9	M	1948	l	·	·	N	e	*B*	*MsNs*	R_1r	+	·	·	−	−	·	−	·	·
III 5–7	II 7, 9	·	·	sb	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
III 8	II 9, 13	M	1942	l	·	·	N	e	A_1	*MsNs*	R_1r	+	·	·	−	−	+	·	·	·
III 9	II 9, 14	M	1952	l	·	·	(N)	e	A_1	*MSNs*	R_1r	−	−	+	−	−	+	+	·	·
							Family 61													
I 1	·	M	1873	l	·	·	Ph ph	e	*O*	*MsMs*	R_1r	−	−	+	−	−	−	·	·	·
I 2	·	F	1882	d 1920	·	·	Ph ph	·	·	·	·	·	·	·	·	·	·	·	·	·
I 3	·	M	1890	l	6.17	240	Ph Ph	e	A_1	*MSMs*	R_1r	+	−	+	−	−	+	+	+	2.1
I 4	·	F	1897	d 1939	·	·	(Ph ph)	·	·	·	·	·	·	·	·	·	·	·	·	·
II 1	I 1, 2	M	1904	l	·	·	ph ph	e	A_1	*MsNs*	R_1R_2	−	−	+	+	−	−	+	−	1.1
II 2	I 1, 2	F	1909	l	·	·	(N)	e	*O*	*MSMs*	R_2r	+	−	+	+	−	−	+	·	·
II 3	I 1, 2	F	1914	l	·	·	(N)	e	·	·	·	·	·	·	·	·	·	·	·	·
II 4	I 1, 2	M	1916	d 1917	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
II 5	I 1, 2	M	1917	l	6.65	*738*	Ph ph	e	A_1	*MSMs*	R_2r	−	+	·	+	+	−	−	−	2.1
II 6	I 1, 2	F	1920	l	·	·	(N)	e	·	·	·	·	·	·	·	·	·	·	·	·
II 7	I 3, 4	F	1920	l	8.35	*914*	Ph ph	e	A_1	*MsNs*	R_1R_1	+	−	·	−	−	−	+	+	1.1
II 8–10	I 3, 4	·	·	l	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
II 11	I 3, 4	·	1939	misc.	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
III 1	II 5, 7	F	1939	l	·	·	N	e	A_1	*MSMs*	R_1R_2	+	−	·	−	+	−	+	·	·
III 2*	II 5, 7	F	1942	l	·	·	ph ph	e	A_1	*MsNs*	R_1R_2	−	−	+	−	−	−	·	·	·
III 3	II 5, 7	M	1945	l	·	·	N	e	*A*	·	·	·	·	·	·	·	·	+	·	·
III 4	II 5, 7	M	1947	l	·	·	N	e	*A*	·	·	·	·	·	·	·	·	+	·	·

† Before correction for dose.

APPENDIX II—*Continued*

1 Ped. no.	2 Parents	3 Sex	4 Year of birth	5 Living or dead	6 Dose G	7 4 hr. phe. μM/l.	8 Class.	9 e	10 *ABO*	11 *MNS*	12 *Rh*	13 P	14 Le(a)	15 Le(b)	16 K	17 Lu(a)	18 Fy(a)	19 Sec.	20 Gm(a)	21 Hp
									Family 62											
I 1–7	·	·	·	4l 3d	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
I 8	·	M	1893	l	6.90	444	N	e	*O*	*NsNs*	R_1r	+	+	−	−	−	−	+	2.1
I 9	·	M	1889	l	5.90	546	Ph ph	e	A_1	*NsNs*	R_1r	+	+	−	−	−	−	+	2.2
I 10	·	F	1885	d 1950	·	·	Ph Ph	·	·	·	·	·	·	·	·	·	·	·	·	·
I 11	·	M	1891	l	·	·	(N)	e	*O*	·	·	·	·	·	·	·	·	·	·	·
I 12	·	F	1890	l	·	·	(N)	e	*O*	·	·	·	·	·	·	·	·	·	·	·
II 1	I 9, 10	M	1913	l	·	·	(N)	e	A_1	·	·	·	−	+	·	·	·	+	·	·
II 2	I 9, 10	M	1916	d 1928	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
II 3	I 9, 10	M	1921	l	6.63	*432*	Ph ph	e	A_1	*NsNs*	*rr*	+	+	−	−	−	−	+	2.2
II 4	I 9, 10	F	1925	l	·	·	(N)	e	·	·	·	·	·	·	·	·	·	·	·	·
II 5	I 11, 12	F	1921	l	6.45	*576*	Ph ph	e	*O*	*MsNs*	R_1r	−	−	+	−	−	+	+	1.1
II 6–10	I 11, 12	·	·	·	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
II 11	I 11, 12	M	1929	d inf.	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
III 1*	II 3, 5	M	1945	l	·	·	ph ph	e	A_1	*NsNs*	R_1r	−	−	+	−	−	+	+	2.1
III 2	II 3, 5	M	1947	l	·	·	(N)	e	A_1	*NsNs*	*rr*	+	−	+	−	−	+	+	2.1
III 3	II 5	F	1948	l	·	·	(N)	e	*O*	*MsNs*	R_1R_2	−	−	+	−	−	+	+	1.1
									Family 63											
I 1	·	M	1889	l	6.17	334	(Ph Ph)	e	A_1	*NsNs*	R_1R_2	−	−	+	+	+	+	·	−	2.1
I 2	·	F	1891	l	5.72	666	Ph ph	e	A_2	*MSMs*	*rr*	−	−	+	−	−	+	·	−	2.2
II 1–2	I 1, 2	·	·	d aged 2	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
II 3	I 1, 2	F	1914	l	4.99	948	Ph ph	e	A_1	*MSNs*	R_1r	−	−	+	−	−	+	+	−	2.2
II 4	·	M	·	l	·	·	(Ph Ph)	·	·	·	·	·	·	·	·	·	·	·	·	·

II 5	I 1, 2	M	1915	1	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·	·
II 6	I 1, 2	F	1916	1	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·	·
II 7	I 1, 2	M	1917	1	5.15	231	Ph Ph	e	A_2	*MsNs*	R_1r	−	−	+	+	−	+	+	−	2.1
II 8	I 1, 2	F	1919	1	5.40	984	Ph ph	e	A_2	*MsNs*	R_1r	−	−	+	−	+	+	+	−	2.1
II 9	·	M	·	1	·	·	(Ph Ph)	·	·	·	·	·	·	·	·	·	·	·	·	·	·
II 10	I 1, 2	M	1921	1	5.08	213	Ph Ph	e	A_1	*MSNs*	R_1r	−	−	+	−	+	−	+	−	2.1
II 11	·	F	·	1	·	·	(Ph Ph)	e	·	·	·	·	·	·	·	·	·	·	·	·	·
II 12	I 1, 2	F	1926	1	5.79	*846*	Ph ph	e	A_1	*MsNs*	R_1r	−	−	+	+	−	−	+	−	·
II 13	·	M	1926	1	6.61	*690*	Ph ph	e	A_1	*NSNs*	R_1r	+	−	+	−	−	+	+	·	·
II 14	I 1, 2	F	1927	d 1939	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
II 15	I 1, 2	F	1928	1	4.45	255	Ph Ph	e	A_1	*MSNs*	R_2r	−	−	+	+	−	+	+	−	2.1
II 16	·	M	·	1	·	·	(Ph Ph)	·	·	·	·	·	·	·	·	·	·	·	·	·	·
II 17	I 1, 2	F	1929	1	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·	·
II 18	I 1, 2	M	1930	1	5.83	792	Ph ph	e	A_2	*MSNs*	R_1r	−	−	+	+	+	+	+	−	2.1
II 19	·	F	1932	1	·	·	(Ph Ph)	e	A_1	·	·	·	·	·	·	·	·	·	·	·	·
II 20	I 1, 2	F	1936	1	4.18	171	Ph Ph	e	A_2	*MSNs*	R_1r	−	−	+	+	−	+	+	−	2.1
III 1	II 3, 4	M	·	1	·	·	(N)	e	A_2	·	·	·	·	·	·	·	·	·	·	·	·
III 2	II 3, 4	F	·	1	·	·	(N)	e	A_1	·	·	·	·	·	·	·	·	·	·	·	·
III 3	II 8, 9	F	1944	1	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·	·
III 4	II 8, 9	M	1950	1	·	·	(N)	e	A_2	·	·	·	·	·	·	·	·	·	·	·	·
III 5	II 10, 11	F	1956	1	·	·	(Ph Ph)	e	*O*	*NsNs*	R_2r	−	−	+	−	−	−	+	·	·
III 6	II 10, 11	M	1957	1	·	·	(Ph Ph)	e	*A*	·	·	·	·	·	·	·	·	+	·	·	·
III 7*	II 12, 13	F	1950	1	·	·	ph ph	e	A_2	*MsNs*	R_1R_1	+	−	+	−	−	−	·	+	2.2
III 8	II 12, 13	F	1951	1	·	·	ph ph	e	A_2	*NsNs*	R_1r	+	−	+	−	−	−	+	−	2.1
III 9	II 12, 13	M	1952	1	·	·	N	e	A_1	*NsNs*	*rr*	+	−	+	+	−	+	+	−	2.2
III 10	II 12, 13	F	1954	1	·	·	N	e	A_1	*NsNs*	R_1r	−	−	+	+	−	+	+	·	·
III 11	II 12, 13	F	1957	1	·	·	N	e	A_1	*MsNS*	*rr*	−	−	·	+	−	−	·	·	·
III 12	II 15, 16	F	1950	1	·	·	(Ph Ph)	e	A_2	·	·	·	·	·	·	·	·	·	·	·	·
III 13	II 18, 19	M	1952	1	·	·	(N)	e	A_1	·	·	·	·	·	·	·	·	·	·	·	·
III 14	II 18, 19	M	1953	1	·	·	(N)	e	A_1	·	·	·	·	·	·	·	·	·	·	·	·
III 15	II 18, 19	M	1955	1	·	·	(N)	e	A_1	·	·	·	·	·	·	·	·	·	·	·	·

APPENDIX II—*Continued*

1 Ped. no.	2 Parents	3 Sex	4 Year of birth	5 Living or dead	6 Dose G	7 4 hr. phe. μM/l	8 Class.	9 e	10 *ABO*	11 *MNS*	12 *Rh*	13 P	14 Le(a)	15 Le(b)	16 K	17 Lu(a)	18 Fy(a)	19 Sec.	20 Gm(a)	21 Hp
							Family 64													
I 1	·	M	·	d	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
I 2–3	·	F	·	d inf.	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
I 4	·	M	1881	l	4.72	372	N	e	A_1	*MsMs*	R_1R_1	+	−	−	−	−	+	+	−	2.2
I 5	·	M	1882	l	·	·	(N)	e	·	·	·	·	·	·	·	·	·	·	·	·
I 6	·	F	1885	l	·	·	(N)	e	*O*	·	·	·	·	·	·	·	·	+	·	·
I 7	·	M	1885	l	·	·	(Ph Ph)	e	*A*	·	·	·	·	·	·	·	·	+	·	·
I 8	·	F	1888	l	6.72	378	N	e	A_1	*MsMs*	R_1R_1	+	−	+	−	−	+	+	−	2.2
I 9	·	M	1889	l	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
I 10	·	M	1890	l	5.63	240	Ph Ph	e	*O*	*MsNs*	R_1R_1	+	−	+	+	−	−	+	−	2.1
I 11	·	M	·	d	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
I 12	·	M	·	l	·	·	(N)	e	·	·	·	·	·	·	·	·	·	·	·	·
I 13	·	F	·	l	·	·	(N)	e	·	·	·	·	·	·	·	·	·	·	·	·
II 1	I 6, 7	F	1914	l	·	·	(N)	e	A_1	*MS*	*rr*	+	−	+	−	−	−	+	−	2.1
II 2	I 6, 7	M	1915	l	·	·	(N)	e	A_1	*MS*	*rr*	+	−	+	−	−	−	+	−	2.2
II 3	I 6, 7	F	1917	d 1946	·	·	?ph ph†	·	·	·	·	·	·	·	·	·	·	·	·	·
II 4	I 6, 7	M	1919	l	·	·	(N)	e	*O*	*MsMs*	R_1r	+	−	+	−	−	−	+	·	·
II 5	I 6, 7	M	1920	d 1939	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
II 6	I 6, 7	M	1922	d 1932	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
II 7	I 6, 7	F	1924	l	·	F186	?Ph ph	e	*O*	*MS*	*rr*	+	−	+	+	+	−	+	·	2.1
II 8	I 8, 9	F	1909	d 1928	·	·	N	·	·	·	·	·	·	·	·	·	·	·	·	·
II 9	I 8, 9	F	1913	l	5.58	293	.92 Ph Ph .08 Ph ph	e	A_1	*MsNs*	R_1R_1	+	−	+	−	−	−	+	+	2.2
II 10	I 8, 9	M	1914	sb	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
II 11	I 8, 9	F	1916	l	5.38	231	Ph Ph	e	*O*	*MsNs*	R_1R_1	+	−	+	−	−	−	+	−	2.2
II 12	I 8, 9	F	1918	l	6.95	684	Ph ph	e	*O*	*MsNs*	R_1R_1	+	−	+	−	−	+	+	−	2.2
II 13	I 8, 9	M	1921	l	7.72	411	N	e	*O*	*MsNs*	R_1R_1	+	−	+	−	−	+	+	+	2.2
II 14	I 8, 9	F	1924	l	4.88	588	Ph ph	e	A_1	*MsNs*	R_1R_1	+	−	+	−	−	−	+	+	2.2

II 15	·	M	1915	l	7.22	*660*	Ph ph	e	*O*	*MNS*	R_1r	+	−	+	−	−	+	+	−	1.1
III 1	II 14, 15	M	1948	l	·	·	(N)	e	A_1	*MsNs*	R_1r	+	−	+	−	−	+	+	−	2.1
III 2*	II 14, 15	M	1949	d 1953	·	·	ph ph	e	·	·	·	·	·	·	·	·	·	·	·	·	·
							Family 65														
I 1	·	M	·	d 1929	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·	·
I 2	·	F	1890	l	7.40	306	.89 Ph Ph .11 Ph ph	e	*O*	*MSNs*	R_1r	+	−	+	−	−	+	+	+	2.1
II 1	I 1, 2	M	1916	d 1931	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·	·
II 2	I 1, 2	M	1920	d inf.	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
II 3	I 1, 2	M	1924	d 1930	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
II 4	I 1, 2	M	1926	l	·	·	Ph ph	e	A_1	*NsNs*	R_2r	+	−	+	−	−	+	+	+	2.2
II 5	·	F	1925	l	·	·	Ph ph	e	*O*	*MsNs*	R_1r	−	−	−	−	−	+	+	−	2.1
III 1*	II 4, 5	F	1954	l	·	·	ph ph	e	*O*	*NsNs*	R_2r	+	−	+	−	−	+	+	+	2.2
III 2	II 4, 5	F	1956	l	·	·	(N)	e	*O*	*MsNs*	R_1R_2	−	−	+	?	−	+	+	·	·
							Family 66														
I 1	·	M	1898	l	·	·	(N)	e	A_1	·	·	·	+	−	+	·	·	·	·	·	·
I 2	·	F	1902	l	·	·	(N)	e	A_1	·	·	·	−	+	−	·	·	·	·	·	·
I 3	·	M	1894	d 1930	·	·	(Ph Ph)	·	·	·	·	·	·	·	·	·	·	·	·	·	·
I 4	·	F	1893	l	7.26	900	Ph ph	e	*O*	*MS*	R_0r	+	−	+	−	−	+	·	−	2.1
II 1–8	I 1, 2	·	·	l	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·	·
II 9	·	M	·	l	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·	·
II 10	I 1, 2	F	1927	l	·	·	Ph ph	e	A_1	*MsNS*	R_1r	+	+	−	?	−	−	−	−	2.2
II 11	I 3, 4	M	1922	l	·	·	Ph ph	e	A_1	*MSMs*	R_2r	+	+	−	−	−	+	−	−	2.1
II 12–15	I 3, 4	·	·	l	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·	·
III 1	II 9, 10	F	1946	l	·	·	(N)	e	*O*	*MsNs*	R_1r	+	+	−	?	−	−	−	−	2.1
III 2	II 10, 11	F	1952	l	·	·	(N)	e	*O*	*MsMs*	*rr*	+	+	−	−	−	+	−	−	2.1

† Fits, mental deficiency from $\frac{9}{12}$ old.

APPENDIX II—*Continued*

1 Ped. no.	2 Parents	3 Sex	4 Year of birth	5 Living or dead	6 Dose G	7 4 hr. phe. μM/l.	8 Class.	9 e	10 *ABO*	11 *MNS*	12 *Rh*	13 P	14 Le(a)	15 Le(b)	16 K	17 Lu(a)	18 Fy(a)	19 Sec.	20 Gm(a)	21 Hp
Family 66—Continued																				
III 3	II 10, 11	M	1953	l	·	·	(N)	e	A_1	*MsMs*	R_1r	+	+	−	−	−	+	−	−	2.1
III 4*	II 10, 11	M	1954	l	·	·	ph ph	e	A_1	*MNS*	*rr*	+	+	−	−	−	−	·	−	2.1
III 5	II 10, 11	·	1958	l	·	·	N	e	·	·	·	·	·	·	·	·	·	·	·	·
Family 67																				
I 1	·	M	1896	l	7.81	942	Ph ph	e	A_1	*MsNs*	R_1r	+	−	+	−	+	+	+	+	2.1
I 2	·	F	1887	l	3.95	333	Ph Ph	e	A_1	*MsNs*	R_1R_2	+	+	−	−	−	−	−	+	2.2
II 1	·	M	1912	l	·	·	Ph ph	·	·	·	·	·	·	·	·	·	·	·	·	·
II 2	I 1, 2	F	1920	l	8.01	*924*	Ph ph	e	A_1	*MsNs*	R_2r	+	−	+	−	−	+	+	+	2.2
II 3	I 1, 2	·	1921	misc.	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
III 1*	II 1, 2	M	1948	l	·	·	ph ph	e	A_1	*MNS*	R_2R_2	+	−	+	−	−	+	+	·	·
III 2	II 1, 2	M	1950	l	·	·	ph ph	e	A_1	*MSMs*	R_2R_2	+	+	+	−	−	+	+	·	·
Family 68																				
I 1	·	M	1882	l	5.72	393	N	e	A_2	*MsNs*	*rr*	−	−	+	−	−	+	+	−	2.1
I 2	·	F	1884	l	·	·	(N)	e	A_1	*MSNs*	R_1r	+	−	+	−	−	+	+	−	2.1
II 1	·	M	·	d	·	·	(Ph Ph)	·	·	·	·	·	·	·	·	·	·	·	·	·
II 2	I 1, 2	F	1908	l	5.72	533	Ph ph	e	A_1	*MSMs*	*rr*	+	−	+	−	−	+	+	−	2.2
II 3	I 1, 2	M	1910	l	7.35	533	Ph ph	e	A_2	*MSNs*	R_1r	+	−	+	−	−	+	+	−	2.1
II 4	·	F	1912	l	·	·	(Ph Ph)	e	A_1	*MNS*	R_2r	+	+	−	−	−	−	−	+	2.1
II 5	I 1, 2	M	1915	l	·	·	Ph ph	e	A_2	*NsNs*	*rr*	+	−	+	−	−	+	+	−	2.1
II 6	·	F	1917	l	·	·	Ph ph	e	*O*	*MSMs*	*rr*	+	−	+	−	−	+	+	+	2.2
III 1	II 1, 2	M	1935	l	6.86	363	N	e	A_2	*MS*	*rr*	+	−	+	−	−	+	+	−	2.2
III 2	II 3, 4	F	1939	l	6.90	276	Ph Ph	e	A_2	*MNS*	*rr*	+	−	+	−	−	+	+	+	2.2
III 3	II 3, 4	F	1950	l	·	·	(N)	e	A_2	*MNS*	·	·	−	+	·	·	·	+	·	·
III 4*	II 5, 6	F	1941	l	·	·	ph ph	e	*O*	*MsNs*	*rr*	+	−	+	−	−	+	·	−	2.2

Family 69

I 1	·	M	1882	l	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
I 2	·	F	1885	l	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
II 1	·	F	1914	l	·	·	Ph ph	e	*O*	*MsMs*	R_1r	+	−	·	−	−	+	·	·	·
II 2	I 1, 2	M	1912	l	·	·	Ph ph	e	A_1	*MsMs*	R_1R_2	+	−	·	−	−	+	·	·	·
II 3	I 1, 2	F	1921	l	5.40	732	Ph ph	e	A_1	*MsNs*	R_1R_1	+	−	+	−	−	−	+	−	2.2
III 1*	II 1, 2	M	1939	l	·	·	ph ph	e	A_1	*MsMs*	R_1r	+	−	·	−	−	+	·	·	·
III 2	II 1, 2	·	·	misc.	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
III 3	II 1, 2	F	1943	l	·	·	ph ph	e	*O*	*MsMs*	R_1r	+	−	+	−	−	+	+	−	2.1

Family 70

I 1	·	M	·	·	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
I 2	·	F	·	l	·	·	(N)	e	A_1	·	·	·	−	+	·	·	·	·	·	·
II 1	I 1, 2	F	1926	l	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
II 2	I 1, 2	F	1927	d 1948	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
II 3	I 1, 2	F	1928	l	·	·	(N)	e	A_1	·	·	·	−	+	·	·	·	·	·	·
II 4	I 1, 2	F	1930	l	·	·	Ph ph	e	A_1	*MSNs*	R_1^wr	+	−	·	−	−	−	·	·	·
II 5	·	M	1921	l	·	·	Ph ph	e	*O*	*MsMs*	*rr*	+	−	·	−	−	+	·	·	·
III 1*	II 4, 5	F	1950	l	·	·	ph ph	e	A_1	*MsNs*	*rr*	+	−	·	−	−	+	·	·	·
III 2	II 4, 5	F	1951	l	·	·	ph ph	e	A_1	*MsNs*	R_1^wr	+	−	·	−	−	−	·	·	·

Family 71

I 1	·	M	·	d	·	·	(Ph Ph)	·	·	·	·	·	·	·	·	·	·	·	·	·
I 2	·	F	1881	l	7.11	1224	Ph ph	e	*O*	*NsNs*	R_1R_1	+	−	+	−	+	+	+	−	2.1
II 1	I 1, 2	F	·	l	·	·	(N)	e	*O*	·	·	·	·	·	·	·	·	·	·	·
II 2	I 1, 2	M	·	l	·	·	(N)	e	*O*	·	·	·	·	·	·	·	·	·	·	·
II 3	I 1, 2	F	·	l	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
II 4	I 1, 2	M	·	l	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
II 5	I 1, 2	F	·	l	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
II 6	I 1, 2	M	1910	l	·	·	Ph ph	e	A_2	*NsNs*	R_1R_2	+	−	+	−	+	−	+	+	2.1
II 7	·	F	1911	l	·	·	Ph ph	e	*O*	*MS*	R_1r	+	−	+	−	−	+	+	−	2.1
III 1*	II 6, 7	M	1935	l	·	·	ph ph	e	*O*	*MSNs*	R_1R_1	+	−	+	−	+	+	·	−	2.2

APPENDIX II—*Continued*

1 Ped. no.	2 Parents	3 Sex	4 Year of birth	5 Living or dead	6 Dose G	7 4 hr. phe. μM/l.	8 Class.	9 e	10 *ABO*	11 *MNS*	12 *Rh*	13 P	14 Le(a)	15 Le(b)	16 K	17 Lu(a)	18 Fy(a)	19 Sec.	20 Gm(a)	21 Hp
								Family 72												
I 1	·	M	1894	l	·	·	Ph ph	e	·	·	·	·	·	·	·	·	·	·	·	·
I 2	·	F	1893	l	·	·	Ph ph	e	A_1B	*MsNs*	R_1r	+	·	·	·	·	−	+	·	·
II 1	I 1, 2	M	1915	l	·	·	(N)	e	*B*	*MsNs*	$R''r$	+	·	·	−	−	+	−	+	·
II 2	I 1, 2	F	1919	l	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
II 3	I 1, 2	M	1920	l	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
II 4	I 1, 2	F	1923	l	·	·	ph ph	e	A_1	*MsMs*	R_1R''	+	+	−	−	−	+	−	−	·
II 5	I 1, 2	F	1925	l	·	·	Ph ph	e	A_1	*MsNs*	R_1r	+	+	−	−	−	+	−	+	2.2
II 6	I 1, 2	F	1926	l	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
II 7	·	M	1919	l	·	·	Ph ph	e	*O*	*MsMs*	R_1R_1	+	+	−	−	−	+	−	−	2.1
III 1	II 5, 7	M	1948	l	·	·	(N)	e	*O*	*MsMs*	R_1r	+	+	−	−	−	+	−	−	·
III 2*	II 5, 7	F	1950	l	·	·	ph ph	e	*O*	*MsMs*	R_1r	+	+	−	−	−	+	·	−	2.1
								Family 73												
I 1	·	M	·	d 1949	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
I 2	·	F	1880	·	·	·	(N)	e	*B*	·	·	·	·	·	·	·	·	+	·	·
II 1	I 1, 2	F	1904	d 1920	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
II 2	I 1, 2	F	1908	l	5.95	237	Ph Ph	e	*B*	*MN*	*rr*	+	−	−	−	−	+	+	−	2.2
II 3	I 1, 2	M	1909	d 1920	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
II 4	I 1, 2	F	1910	l	4.68	441	N	e	*O*	*MN*	R_1r	+	−	−	−	−	+	+	+	2.1
II 5	I 1, 2	M	1916	l	7.49	*841*	Ph ph	e	*B*	*MsNs*	R_1r	+	−	·	−	−	+	+	·	·
II 6–10	·	·	·	2l 3d	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
II 11	·	M	1920	·	·	·	(N)	e	*O*	·	·	·	·	·	·	·	·	·	·	·
II 12	·	F	1922	l	5.63	*426*	Ph ph	e	A_1	*NSNs*	R_1R_1	+	−	·	−	−	−	+	·	·
II 13	I 1, 2	M	1918	l	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·

II 14	I 1, 2	F	1923	l	5.04	720	Ph ph	e	*B*	*MN*	*rr*	+	−	+†	−	−	+	+	−	2.2
II 15	·	M	·	l	·	·	(Ph Ph)	·	·	·	·	·	·	·	·	·	·	·	·	·
III 1*	II 5, 12	M	1944	l	·	·	ph ph	e	*B*	*MsNs*	R_1r	+	−	·	−	−	−	·	·	·
III 2	II 5, 12	M	1949	l	·	·	ph ph	e	*B*	*NsNs*	R_1r	+	·	·	−	−	−	·	·	·
III 3	II 14, 15	F	1937	l	5.13	420	N	e	*O*	*MsNs*	R_1r	+	−	+	−	−	+	·	−	2.1
III 4	II 14, 15	F	1941	l	·	·	(N)	e	*B*	·	·	·	·	·	·	·	·	·	·	·
							Family 74													
I 1	·	M	·	l	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
I 2	·	F	·	l	·	·	(N)	e	A_1	*NsNs*	*rr*	−	+	−	−	−	+	·	·	·
II 1	I 1, 2	F	·	l	·	·	N	e	A_1	*NsNs*	R_1r	−	−	+	−	−	+	·	−	2.1
II 2	I 1, 2	M	1919	l	·	·	Ph ph	e	A_1	*NsNs*	*rr*	+	−	+	−	−	+	+	+	1.1
II 3	·	F	1921	l	·	·	Ph ph	e	A_1	*NsNs*	R_1R_2	+	+	−	−	−	−	−	−	2.1
III 1*	II 2, 3	F	1945	l	·	·	ph ph	e	A_1	*NsNs*	R_1r	+	+	−	−	−	+	−	+	2.1
III 2	II 2, 3	M	1948	l	·	·	(N)	e	*O*	·	·	·	·	·	·	·	·	−	·	·
							Family 75													
I 1	·	M	1864	d 1952	·	·	Ph ph‡	·	·	·	·	·	·	·	·	·	·	·	·	·
I 2	·	F	1871	d 1934	·	·	Ph ph‡	·	·	·	·	·	·	·	·	·	·	·	·	·
I 3	·	M	1865	d 1948	·	·	Ph ph	·	·	·	·	·	·	·	·	·	·	·	·	·
I 4	·	F	1862	d 1947	·	·	Ph Ph	·	·	·	·	·	·	·	·	·	·	·	·	·
II 1	I 1, 2	F	1892	l	6.67	1140	Ph ph	e	*O*	*MNS*	R_1R_1	−	−	+	−	−	−	+	+	2.1
II 2	I 1, 2	F	1894	d 1909	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
II 3	I 1, 2	M	1896	l	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
II 4	I 1, 2	F	1899	l	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
II 5	I 1, 2	M	1902	d 1923	·	·	ph ph‡	·	·	·	·	·	·	·	·	·	·	·	·	·
II 6	I 1, 2	F	1906	l	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
II 7	I 3, 4	M	1886	l	5.72	347	N	e	A_1	*MS*	R_1r	+	−	+	−	−	+	+	+	2.2

† Le(b) present in saliva, red cells not tested.

‡ II 5 Imbecile, fits, fair hair. Probably phenylketonuric especially since sib II 1 is heterozygote.

APPENDIX II—*Continued*

1 Ped. no.	2 Parents	3 Sex	4 Year of birth	5 Living or dead	6 Dose G	7 4 hr. phe. μM/l.	8 Class.	9 e	10 *ABO*	11 *MNS*	12 *Rh*	13 P	14 Le(a)	15 Le(b)	16 K	17 Lu(a)	18 Fy(a)	19 Sec.	20 Gm(a)	21 Hp
Family 75—Continued																				
II 8	I 3, 4	M	1888	l	5.99	666	Ph ph	e	A_1	*MNS*	R_1r	+	−	+	−	−	+	+	+	2.2
II 9	·	F	1888	d 1945	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
II 10	I 3, 4	M	1889	d 1948	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
II 11	I 3, 4	M	1891	l	·	·	Ph ph	e	A_1	*MsNs*	R_1R_1	+	−	+	·	−	·	+	·	·
II 12	·	F	1892	l	·	·	Ph Ph	e	*O*	*MS*	R_1r	+	−	+	·	−	·	+	·	·
II 13	I 3, 4	F	1892	l	·	· ·	(N)	e	·	·	·	·	·	·	·	·	·	·	·	·
II 14	I 3, 4	F	1893	d 1895	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
II 15	I 3, 4	F	1895	d	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
II 16	I 3, 4	F	1898	l	5.38	354	N	e	A_1	*MS*	R_1r	−	−	+	−	−	+	+	−	2.2
II 17	I 3, 4	F	1900	d	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
II 18	I 3, 4	M	1903	l	7.22	375	N	e	A_1	*MS*	*rr*	+	−	+	−	−	+	+	+	2.2
III 1	II 8, 9	F	1915	l	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
III 2	II 8, 9	M	1916	l	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
III 3	II 8, 9	F	1918	d 1932	·	·	?ph ph	·	·	·	·	·	·	·	·	·	·	·	·	·
III 4	II 8, 9	M	·	l	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
III 5	II 8, 9	M	1924	l	·	·	(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
III 6	II 11, 12	M	1917	l	6.92	336	N	e	*O*	*MSNs*	R_1R_1	+	−	+	−	−	+	+	−	2.1
III 7	II 11, 12	F	1919	l	·	·	(N)	e	·	·	·	·	·	·	·	·	·	·	·	·
III 8	II 11, 12	F	1924	l	5.97	269	Ph Ph	e	*O*	*MSNs*	R_1R_1	+	−	+	−	−	+	+	+	2.2
III 9	II 11, 12	M	1926	l	5.90	300	.10 Ph ph .90 Ph Ph	e	*O*	*MSNs*	R_1r	+	−	+	−	−	+	+	−	2.2
III 10	II 11, 12	F	1928	l	·	·	(N)	e	·	·	·	·	·	·	·	·	·	·	·	·
III 11	II 11, 12	F	1930	l	·	·	Ph ph	e	A_1	*MSNs*	R_1r	+	−	+	−	−	+	+	+	2.2
III 12	·	M	·	l	·	·	Ph ph	e	*O*	*MsMs*	R_1^wr	+	−	+	−	−	+	+	+	2.1
IV 1	III 11, 12	M	1950	l	·	·	(N)	e	*O*	·	·	·	·	·	·	·	·	+	·	·
IV 2*	III 11, 12	M	1951	l	·	·	ph ph	e	A_1	*MsNs*	R_1r	+	−	+	−	−	+	+	+	2.1
IV 3	III 11, 12	M	1954	l	·	·	(N)	e	*A*	·	·	·	·	·	·	·	·	+	·	·
IV 4	III 11, 12	M	1956	l	·	·	ph ph	e	A_1	*MsNs*	$R_1^wR_1$	+	−	+	−	−	+	·	+	2.2

IV 4	III 11, 12	M	1956	1	.	.	ph ph	e	A_1	*MsNs*	$R_1^wR_1$	+	−	+	−	−	+	·	+	2.2

Family 76

I 1		M	1893	d 1957			Ph ph	e	A_1	*MsNs*	*rr*	+	−	·	−	−	+	+	·	·
I 2		F	1895	1			Ph ph	e	A_1	*MNS*	R_1r	+	+	−	−	−	+	−	−	2.2
II 1		M	1917	1			(N)	·	·	·	.	.	.	.	.	.	.	.	.	.	.
II 2		F	1919	1			(N)	·	·	·	.	.	.	.	.	.	.	.	.	.	.
II 3		M	1923	1			(N)	e	A_1	*MNS*	*rr*	+	−	·	−	−	+	+	·	·
II 4		F	1935	1			(N)	e	A_1	*MNS*	R_1r	+	+	·	−	−	−	−	·	·
II 5*		F	1937	1			ph ph	e	A_1	*MSMs*	R_1r	+	+	·	−	−	+	−	·	·

Family 77

I 1		M	1901	1			Ph ph	e	*O*	*NS*	$R''r(D^u)$	+	−	+	−	−	+	+	+	2.1
I 2		F	1906	1			Ph ph	e	*O*	*MNS*	R_1R_1	−	−	+	−	−	+	+	−	2.1
II 1		F	1926	1			(N)	e	*O*	*NS*	R_1R''	+	−	+	−	−	+	+	+	2.1
II 2		M	1928	1			(N)	e	*O*	*MNS*	R_1r	−	+	−	−	−	+	−	+	1.1
II 3		·	·	misc.			·	·	·	·	.	.	.	.	.	.	.	.	.	.	.
II 4*		M	1943	1			ph ph	e	*O*	*MNS*	R_1r	+	+	−	−	−	+	·	+	2.2

Family 78

I 1		M	1893	1			Ph ph	e	*O*	*MS*	R_2r	+	−	+	−	+	+	+	+	2.1
I 2		F	1896	1			Ph ph	e	*O*	*MNS*	R_2r	+	−	+	−	−	+	+	+	2.2
II 1*		F	1914	1			ph ph	e	*O*	*MS*	*rr*	+	−	+	−	+	+	+	+	2.1
II 2		F	1915	1			ph ph	e	*O*	*MNS*	R_2r	+	−	+	−	+	+	+	+	1.1

Family 79

I 1		M	1915	1			Ph ph	e	*O*	*MSMs*	R_1r	−	−	+	−	−	+	+	+	2.1
I 2		F	1919	1			Ph ph	e	*O*	*MSMs*	R_1r	+	+	−	+	−	−	−	+	2.2
II 1		·	·	misc.			·	·	·	·	.	.	.	.	.	.	.	.	.	.	.
II 2*		M	1947	1			ph ph	e	·	·	.	.	.	.	.	.	.	.	.	.	.
II 3		F	1949	1			(N)	e	*O*	*MS*	R_1R_1	+	−	+	−	−	+	+	+	·

APPENDIX II—*Continued*

1 Ped. no.	2 Parents	3 Sex	4 Year of birth	5 Living or dead	6 Dose G	7 4 hr. phe. μM/l.	8 Class.	9 e	10 *ABO*	11 *MNS*	12 *Rh*	13 P	14 Le(a)	15 Le(b)	16 K	17 Lu(a)	18 Fy(a)	19 Sec.	20 Gm(a)	21 Hp
Family 79—Continued																				
II 4 }		F	1952	l			(N)	e	*O*	*MS*	R_1R_1	+	−	+	+	−	+	+	+	2.1
II 5 }		F	1952	l			(N)	e	*O*	*MsMs*	R_1r	+	+	−	+	−	−	−	+	2.2
II 6		F	1955	l			(N)	e	*O*	*MS*	R_1r	+	+	−	+	−	−	−	·	·
II 7		M	1957	l			(N)	e	*O*	*MsMs*	*rr*	+	−	+	+	−	−	+	·	·
Family 80																				
I 1		M	1890	d 1955			Ph ph	.	.	.	.	.	.	.	.	.	.	.	.	.
I 2		F	1895	l			Ph ph	e	*O*	*MSMs*	R_1R_2	+	+	−	−	−	−	−	2.2
II 1		F	1916	l			(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
II 2		M	1919	l			(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
II 3*		F	1921	l			phph	e	*O*	*MsNs*	R_1r	−	−	+	−	−	+	+	+	2.2
II 4		F	1924	l			(N)	e	*O*	*MsNs*	R_2R_2	+	−	+	−	−	+	+	+	2.2
II 5		F	1925	l			ph ph	e	*O*	*MNS*	R_2R_2	−	−	+	−	−	−	+	−	2.1
II 6		M	1926	l			(N)	e	*O*	*MNS*	R_2r	+	−	+	−	−	+	+	−	2.2
II 7		M	1930	l			(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
II 8		M	1933	l			(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
II 9		·	1940	misc.			·	·	·	·	·	·	·	·	·	·	·	·	·	·
Family 81																				
I 1		M	1916	l			Ph ph	e	*O*	*MsNs*	R_2r	−	−	+	−	−	−	+	+	2.2
I 2		F	1915	l			Ph ph	e	*O*	*NsNs*	R_1r	+	−	−	−	−	+	−	·	·
II 1*		M	1942	l			ph ph	e	*O*	*NsNs*	*rr*	+	−	+	−	−	+	·	·	·
II 2		F	1948	l			(N)	e	*O*	*MsNs*	R_2r	−	−	·	−	−	−	+	·	·
II 3		F	1949	l			ph ph	e	*O*	*NsNs*	R_2r	+	−	·	−	−	−	+	·	·

Family 82

I 1		M	1915	l			Ph ph	·	·	·	·	·	·	·	·	·	·	·	·	·
I 2		F	1916	l			Ph ph	e	A_1	*MNS*	R_1r	+	−	+	−	−	+	+	+	2.2
II 1*		F	1941	l			ph ph	e	*B*	*NS*	*rr*	+	−	+	−	−	+	+	−	2.2
II 2		F	1946	l			(N)	e	*O*	*MNS*	R_1r	+	−	+	−	+	+	+	+	2.2
II 3		F	1947	l			(N)	e	*O*	*MNS*	R_1R_1	−	−	+	−	+	+	+	−	2.2
II 4		·	·	misc.			·	·	·	·	·	·	·	·	·	·	·	·	·	·	·

Family 83

I 1		M	1886	d 1953			Ph ph	·	·	·	·	·	·	·	·	·	·	·	·	·
I 2		F	1888	d 1955			Ph ph	·	·	·	·	·	·	·	·	·	·	·	·	·
II 1*		M	1913	l			ph ph	e	*O*	*MS*	R_1r	+	+	−	−	−	+	−	+	2.1
II 2		M	1916	l			(N)	e	*O*	*MsNs*	*rr*	+	−	+	−	−	−	+	−	1.1
II 3		M	1924	l			(N)	e	*O*	*MNS*	*rr*	−	−	+	−	−	−	+	+	2.2

Family 84 First cousin mating, father of I 1 being sib of mother of I 2

I 1		M	1879	d 1955			Ph ph	·	·	·	·	·	·	·	·	·	·	·	·	·
I 2		F	1888	l			Ph ph	e	A_1	*MsNs*	R_1R_1	+	−	+	−	−	+	+	+	2.1
II 1		M	·	sb			·	·	·	·	·	·	·	·	·	·	·	·	·	·
II 2		F	1908	l			(N)	e	A_1	*MsNs*	R_1R_1	−	+	−	+	−	+	·	·	·
II 3		M	1909	d 1911			·	·	·	·	·	·	·	·	·	·	·	·	·	·
II 4		F	1913	l			(N)	e	A_1	*MsNs*	R_1R_1	+	+	−	+	−	+	·	·	·
II 5		M	1915	d 1933			?ph ph†	·	·	·	·	·	·	·	·	·	·	·	·	·
II 6		F	1922	l			(N)	e	A_1	·	R_1R_1	+	+	·	−	−	+	·	·	·
II 7		M	1923	l			ph ph	e	A_1	*MsMs*	R_1R_1	+	+	·	−	−	+	−	·	·
II 8		F	1925	d 1933			?ph ph‡	·	·	·	·	·	·	·	·	·	·	·	·	·
II 9*		F	1927	l			ph ph	e	A_1	*MsNs*	R_1r	+	−	·	+	−	+	·	·	·
II 10		M	1929	d 1931			·	·	·	·	·	·	·	·	·	·	·	·	·	·

† Died in mental deficiency institution.
‡ Backward (special school).

APPENDIX II—*Continued*

1 Ped. no.	2 Parents	3 Sex	4 Year of birth	5 Living or dead	6 Dose G	7 4 hr. phe. μM/l.	8 Class.	9 e	10 *ABO*	11 *MNS*	12 *Rh*	13 P	14 Le(a)	15 Le(b)	16 K	17 Lu(a)	18 Fy(a)	19 Sec.	20 Gm(a)	21 Hp
									Family 85											
I 1		M	1893	l			Ph ph	e	*A*	*MSNs*	R_0r	+	−	+	−	−	+	+	+	2.2
I 2		F	·	·			Ph ph	·	·	·	·	·	·	·	·	·	·	·	·	·
II 1*		M	1915	l			ph ph	e	A_*	*NsNs*	R_1r	+	−	+	−	−	+	+	+	2.2
II 2		F	1920	l			(N)	e	A_*	*MSNs*	R_1r	+	−	+	−	−	+	+	−	2.2
II 3		F	1923	l			(N)	e	A_*	*MsNs*	R_1r	−	−	+	−	−	+	+	+	2.2
II 4		F	1934	l			ph ph	e	*O*	*MSNs*	R_1r	+	−	+	−	−	+	·	−	2.2
									Family 86											
I 1		M	1923	l			Ph ph	e	*O*	*MSNs*	R_1R_1	+	−	·	−	−	+	·	·	·
I 2		F	1929	l			Ph ph	e	A_1	*MsMs*	rr	−	+	·	−	−	−	−	+	2.2
II 1*		M	1949	l			ph ph	e	*O*	*MSMs*	R_1r	−	+	−	−	−	+	+	·	·
II 2		M	1950	l			(N)	e	*O*	*MsNs*	R_1r	−	−	+	−	−	+	+	+	2.2
II 3		F	1954	l			(N)	e	A_1	*MSMs*	R_1r	−	+	−	−	−	+	−	·	·
II 4		·	1958	l			N	·	·	·	·	·	·	·	·	·	·	·	·	·
									Family 87											
I 1		M	·	l			Ph ph	e	*O*	·	·	·	·	·	·	·	·	·	·	·
I 2		F	·	l			Ph ph	e	*O*	·	·	·	·	·	·	·	·	·	·	·
II 1		M	1944	l			(N)	e	*O*	·	·	·	·	·	·	·	·	·	·	·
II 2		F	1948	l			(N)	e	*O*	·	·	·	·	·	·	·	·	·	·	·
II 3		F	1949	l			(N)	e	*O*	·	·	·	·	·	·	·	·	·	·	·
II 4		M	1950	l			(N)	e	*O*	·	·	·	·	·	·	·	·	·	·	·
II 5		M	1951	l			(N)	e	*O*	·	·	·	·	·	·	·	·	·	·	·

II 6*		F	1952	l			ph ph	e	*O*	·	·	·	·	·	·	·	·	·	·	·	·
II 7		F	1954	l			ph ph	e	*O*	·	·	·	·	·	·	·	·	·	·	·	·
II 8		F	1955	l			ph ph	e	*O*	·	·	·	·	·	·	·	·	·	·	·	·
II 9		F	1956	l			(N)	e	*O*	·	·	·	·	·	·	·	·	·	·	·	·
II 10		M	1957	l			ph ph	e	*O*	·	·	·	·	·	·	·	·	·	·	·	·
							Family 88														
I 1		M	1879	l			Ph ph	e	*O*	*NsNs*	R_2r	+	−	+	−	−	−	+	−	2.2
I 2		F	1881	l			Ph ph	e	A_1	*MS*	R_1r	+	−	+	−	−	−	+	+	2.1
II 1		F	1909	l			(N)	e	*O*	*MSNs*	R_1R_2	+	−	+	−	−	−	+	−	2.1
II 2		M	1911	l			(N)	e	*O*	*MSNs*	R_1r	+	−	+	−	−	−	+	−	2.2
II 3*		M	1913	l			ph ph	e	A_1	*MSNs*	R_2r	+	−	+	−	−	−	+	−	2.1
II 4		F	1916	d 1921			?ph ph	·	·	·	·	·	·	·	·	·	·	·	·	·	·
							Family 89														
I 1		M	1916	l			Ph ph	e	*O*	*NsNs*	R_1r	+	+	−	−	−	+	·	+	2.2
I 2		F	1922	l			Ph ph	e	*O*	*NsNs*	R_1r	+	+	−	−	−	+	·	+	1.1
II 1*		F	1952	l			ph ph	e	*O*	*NsNs*	*rr*	+	+	·	−	−	+	·	·	·
							Family 90														
I 1		M	·	d			Ph ph	·	·	·	·	·	·	·	·	·	·	·	·	·	·
I 2		F	·	d			Ph ph	·	·	·	·	·	·	·	·	·	·	·	·	·	·
II 1		F	·	d			·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
II 2		·	·	d			·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
II 3		M	·	l			(N)	e	*O*	*MS*	R_1r	+	+	·	−	−	+	−	·	·
II 4*		M	1903	l			ph ph	e	A_1	*MsNs*	R_1r	+	−	+	−	−	+	·	+	2.1
II 5		F	1905	l			(N)	e	A_2	*MsNs*	R_1r	+	−	·	+	−	+	+	·	·
II 6		F	1908	l			(N)	e	*O*	*MS*	R_1r	+	+	·	+	−	+	·	·	·
II 7		F	·	l			(N)	e	A_1	*MS*	R_1r	·	·	·	+	·	+	·	·	·

A* Intermediate between A_1 and A_2.

APPENDIX II—*Continued*

1 Ped. no.	2 Parents	3 Sex	4 Year of birth	5 Living or dead	6 Dose G	7 4 hr. phe. μM/l.	8 Class.	9 e	10 *ABO*	11 *MNS*	12 *Rh*	13 P	14 Le(a)	15 Le(b)	16 K	17 Lu(a)	18 Fy(a)	19 Sec.	20 Gm(a)	21 Hp
									Family 91											
I 1		M	·	l			Ph ph	·	·	·	·	·	·	·	·	·	·	·	·	·
I 2		F	1926	l			Ph ph	e	A_1	*MsNs*	R_1R_2	+	−	+	−	−	+	·	+	2.1
II 1		M	1952	l			(N)	e	A_1	*MNS*	R_2R_2	+	−	+	−	−	+	·	·	·
II 2*		F	1952	l			ph ph	e	A_1	*NSNs*	R_2R_2	+	−	+	−	−	+	+	+	2.1
									Family 92											
I 1		M	1905	l			Ph ph	e	*O*	*MS*	R_1R_2	+	−	·	−	−	−	+	·	·
I 2		F	1911	l			Ph ph	e	*O*	*MS*	R_1R_2	−	−	·	−	−	+	+	·	·
II 1*		M	1935	l			ph ph	e	*O*	*MS*	R_1R_2	+	−	+	−	−	+	·	+	2.1
II 2		M	1938	l			(N)	e	*O*	*MS*	R_2R_2	+	−	·	−	−	+	+	·	·
II 3		F	1942	l			ph ph	e	*O*	*MS*	R_1R_1	+	−	·	−	−	+	·	·	·
II 4		M	1948	l			(N)	e	*O*	*MS*	R_1R_2	+	−	·	−	−	+	+	·	·
									Family 93											
I 1		M	1902	l			Ph ph	e	A_1	*MNS*	R_1r	+	−	+	−	−	+	·	+	2.2
I 2		F	1913	l			Ph ph	e	A_1	*MNS*	R_2	+	+	−	−	−	+	·	·	·
II 1		M	1946	l			(N)	e	A_1	*MNS*	R_1R_2	+	+	−	−	−	+	·	−	2.2
II 2*		F	1949	l			ph ph	e	A_1	*MS*	R_2r	+	+	−	−	−	+	·	+	2.2
									Family 94											
I 1		M	·	l			Ph ph	e	*B*	*M*	R_1r	+	·	·	·	·	·	·	·	·
I 2		F	·	l			Ph ph	e	*O*	*MN*	R_1R_1	+	·	·	·	·	·	·	·	·
II 1		F	·	l			(N)	e	*B*	*M*	R_1R_1	+	·	·	·	·	·	·	·	·
II 2*		F	·	l			ph ph	e	*O*	*M*	R_1R_1	+	·	·	·	·	·	·	·	·

Family 95

I 1		M	1899	l			Ph ph	e	*O*	*NsNs*	R_1r	+	−	+	−	−	+	+	+	2.2
I 2		F	1901	l			Ph ph	e	*O*	*MS*	R_1r	+	−	−	−	−	+	−	+	1.1
II 1		F	1928	l			(N)	·	·	·	·	·	·	·	·	·	·	·	·	·	·
II 2*		M	1932	l			ph ph	e	*O*	*MSNs*	R_1r	+	−	·	−	−	+	+	+	2.1
II 3		F	1934	l			(N)	e	*O*	*MSNs*	R_1R_1	−	−	−	−	−	+	+	+	2.1
II 4		M	1938	l			(N)	e	*O*	*MSNs*	R_1r	+	−	−	−	−	+	+	+	2.1

Family 96

I 1		M	·	l			Ph ph	e	*O*	*MN*	R_2r	−	·	·	·	·	·	·	·
I 2		F	·	l			Ph ph	e	*O*	*M*	R_1R_1	−	·	·	·	·	·	·	·
II 1*		M	·	l			ph ph	e	*O*	*MN*	R_1R_2	−	·	·	·	·	·	·	·
II 2		M	·	l			(N)	e	*O*	*M*	R_1r	−	·	·	·	·	·	·	·
II 3		F	·	l			ph ph	e	*O*	*M*	R_1R_2	−	·	·	·	·	·	·	·
II 4		F	·	l			(N)	e	*O*	*MN*	R_1R_2	−	·	·	·	·	·	·	·
II 5		F	·	l			(N)	e	*O*	*MN*	R_1r	−	·	·	·	·	·	·	·

Family 97

I 1		M	·	d			Ph ph	·	·	·	·	·	·	·	·	·	·	·	·
I 2		F	·	d			Ph ph	·	·	·	·	·	·	·	·	·	·	·	·
II 1		M	1895	d			?ph ph	·	·	·	·	·	·	·	·	·	·	·	·
II 2		M	1897	d			(N)	·	·	·	·	·	·	·	·	·	·	·	·
II 3		M	·	l			(N)	e	A_1	*MNS*	*rr*	+	−	·	−	−	+	·	·	·
II 4		·	·	sb			(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
II 5		F	·	d			?ph ph	·	·	·	·	·	·	·	·	·	·	·	·	·
II 6*		F	·	l			ph ph	e	A_1	*MNS*	R_1r	+	−	+	−	−	+	+	·	·
II 7		F	·	d			(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
II 8		F	·	l			(N)	e	A_1	*MsNs*	R_1r	+	−	+	−	−	+	+	·	·

APPENDIX II—*Concluded*

1 Ped. no.	2 Parents	3 Sex	4 Year of birth	5 Living or dead	6 Dose G	7 4 hr. phe. μM/l.	8 Class.	9 e	10 *ABO*	11 *MNS*	12 *Rh*	13 P	14 Le(a)	15 Le(b)	16 K	17 Lu(a)	18 Fy(a)	19 Sec.	20 Gm(a)	21 Hp
							Family 98													
I 1		M	1914	l			Ph ph	e	A_1	*MsNs*	R_1r	+	−	·	−	−	+	·	·	·
I 2		F	1917	l			Ph ph	e	*O*	*MSMs*	R_1r	−	−	+	−	−	+	·	·	·
II 1		M	·	l			(N)	e	*O*	*MSNs*	R_1R_1	+	−	+	−	−	+	+	·	·
II 2*		M	·	l			ph ph	e	*O*	*MSNs*	R_1R_1	+	−	+	−	−	−	·	·	·
II 3		F	·	l			ph ph	e	*O*	*MsMs*	R_1R_1	+	−	·	−	−	−	+	·	·
							Family 99													
I 1		M	·	d			Ph ph	·	·	·	·	·	·	·	·	·	·	·	·	·
I 2		F	·	d 1904			Ph ph	·	·	·	·	·	·	·	·	·	·	·	·	·
II 1		M	1898	l			(N)	·	·	·	·	·	·	·	·	·	·	·	·	·
II 2*		F	1899	l			ph ph	e	*B*	*MS*	R_1r	+	+	−	−	−	−	+	2.2	
II 3		F	1904	l			N	e	A_1	*MNS*	R_1r	+	−	+	+	−	+	+	2.1	
II 4		M	·	l			(N)	·	·	·	·	·	·	·	·	·	·	·	·	·	·
							Family 100													
I 1		M	1896	l			Ph ph	e	*O*	*MS*	*rr*	−	−	+	−	−	+	−	2.2	
I 2		F	1902	l			Ph ph	e	*O*	*MNS*	R_2r	+	−	+	−	−	+	+	+	2.2
II 1*		M	1925	l			ph ph	e	*O*	*MNS*	*rr*	−	−	+	−	−	+	+	+	2.2
II 2		F	1928	l			ph ph	e	*O*	*MNS*	*rr*	−	+	−	−	−	+	−	+	2.2
							Family 101													
I 1		M	·	l			Ph ph	e	*O*	*MN*	R_1r	+	·	·	·	·	·	+	·	·
I 2		F	·	l			Ph ph	e	*O*	*MN*	*rr*	+	·	·	·	·	·	+	·	·
II 1		F	·	l			(N)	e	*O*	*N*	·	+	·	·	·	·	·	+	·	·
II 2*		F	·	l			ph ph	e	*O*	*N*	·	+	·	·	·	·	·	+	·	·

True Idiopathic Hypoparathyroidism as a Sex-Linked Recessive Trait

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TRUE IDIOPATHIC HYPOPARATHYROIDISM (THP) is a relatively rare disease, as evidenced by the fact that only 58 acceptable cases had been reported in the literature up to 1960 (Bronsky, 1958; Bergstrand, 1958; Buchs, 1955, 1957; Axelrod, 1950; Naef, 1959; Soper, 1957; Symon, 1959). Familial occurrence of THP is so uncommon that this disease is not usually thought of as being hereditary. Cases of THP presenting with hypocalcemic convulsions during the neonatal period or at ages less than one year are particularly unusual.

It is the purpose of this paper to present two cases of neonatal THP occurring in siblings, together with evidence suggesting that there may be more than one type of THP, and that one type is transmitted as a sex-linked recessive trait.

The requirements for acceptance of a case as THP were first set forth by Drake (1939) for idiopathic hypoparathyroidism and subsequently were modified by Bronsky (1958) to exclude pseudohypoparathyroidism. The criteria are:

1) low serum calcium
2) high serum phosphorus
3) chronic tetany or convulsions
4) absence of roentgenologic signs of rickets or osteomalacia
5) absence of renal insufficiency, steatorrhea, chronic diarrhea, and alkalosis
6) absence of physical characteristics of pseudohypoparathyroidism, such as brachydactyly, dwarfing, or subcutaneous calcium deposition.

Excluded from the category of THP were cases showing failure to respond to parathormone by increased urinary phosphorus excretion, and cases with meager or questionable clinical findings having demonstrable parathyroid glands on biopsy or post-mortem examination. However, in cases with clear-cut clinical findings it was not required for inclusion as THP that response to parathormone be tested, or that biopsy or post-mortem examination be made. Some cases which may well have been THP had to be excluded because the information given in the reports was insufficient to permit distinction between THP and pseudohypoparathyroidism.

CASE REPORTS

Case No. 1. C.B., a white male, was first seen at the age of 7 weeks because of recurrent generalized convulsions during the previous 24 hours. Birth history

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was normal, with no trauma, cyanosis or jaundice, and the patient had been on breast feedings since birth. The child was afebrile, and the physical examination was negative except for convulsive state. A lumbar puncture revealed normal spinal fluid, and subdural taps were negative. Serum calcium was 3.0 mEq./L. (normal: 4.5 to 5.5 mEq./L.), and serum phosphorus was 3.7 mEq./L. (normal: 2.5 to 3.5 mEq./L.). Oral calcium and anticonvulsants were started. Serum Ca rose to 3.8 mEq./L., and major seizures subsided, but the child continued to have intermittent twitchings of the face and the extremities. Excessive mucus was noted to drool continuously from the mouth. On one occasion difficult respirations with inspiratory stridor were noted.

Against advice, the child was signed out of the hospital by his parents, but was returned at the age of 2½ months because twitchings and major seizures continued to occur. On this second admission serum Ca was consistently low (2.8 to 3.3 mEq./L.), and serum P consistently high (4.1 to 6.1 mEq./L.). The Sulkowitch test on the urine was repeatedly negative. On several occasions the convulsions responded temporarily to intravenous calcium, but recurred after a few hours. The child was treated for one week with large daily doses of Vitamin D, oral calcium, and aluminum hydroxide gel, after which the convulsions and twitchings subsided, although serum Ca and P failed to return to normal.

The patient was discharged from the hospital at the age of 3½ months and returned for re-admission at the age of 4½ months because of an upper respiratory infection. Bilateral cataracts were noted at this time. The child had had no further convulsions, although he was receiving no medication other than added calcium. Serum Ca on admission was 4.8 mEq./L., P was 4.7 mEq./L. During hospitalization, the serum Ca again dropped to low levels (3.2–3.3 mEq./L.), the serum P rose (6.5–7.0 mEq./L.), and convulsions recurred. Vitamin D was restarted, Ca and P returned to more nearly normal levels, and the convulsions ceased.

During the subsequent 2½ years this child had eight additional hospital admissions, usually for convulsions. Chvostek and Trousseau signs were always negative, even when the serum Ca was low. X-rays of skull, long bones, and hands were normal. Intravenous pyelograms were negative on two occasions. Repeated urinalyses, BUN determinations, and two urea clearance tests were normal. Blood sugars, serum electrolytes (Na, Cl, K, and CO_2), alkaline phosphatase, and serum proteins were consistently within normal limits. Two Ellsworth-Howard tests, run with simultaneous normal controls of similar age and weight, showed a definite increase in urine phosphate in response to parathormone. There was no chronic diarrhea or steatorrhea, and the child had no monilial infections.

Oral Vitamin D and calcium have been continued up to the present. The daily dose of Vitamin D required to maintain serum Ca and P at relatively normal levels has fluctuated, without apparent reason, between 50,000 and 250,000 units. On therapy, serum Ca has ranged between 3.2–5.8 mEq./L., with

most of the values falling between 4.4–5.4 mEq./L. Serum P has ranged between 2.5–7.0 mEq./L. The patient had one episode of hypercalcemia (Ca 8.3 mEq./L., P 1.1 mEq./L., with 4+ urine Sulkowitch), which responded to decrease in dosage of Vitamin D. He is at present maintained on 50,000 units daily, with added oral calcium, aluminum hydroxide gel, and a high Ca/P ratio milk. The most recent serum Ca was 3.9 mEq./L., and serum P 3.4 mEq./L.

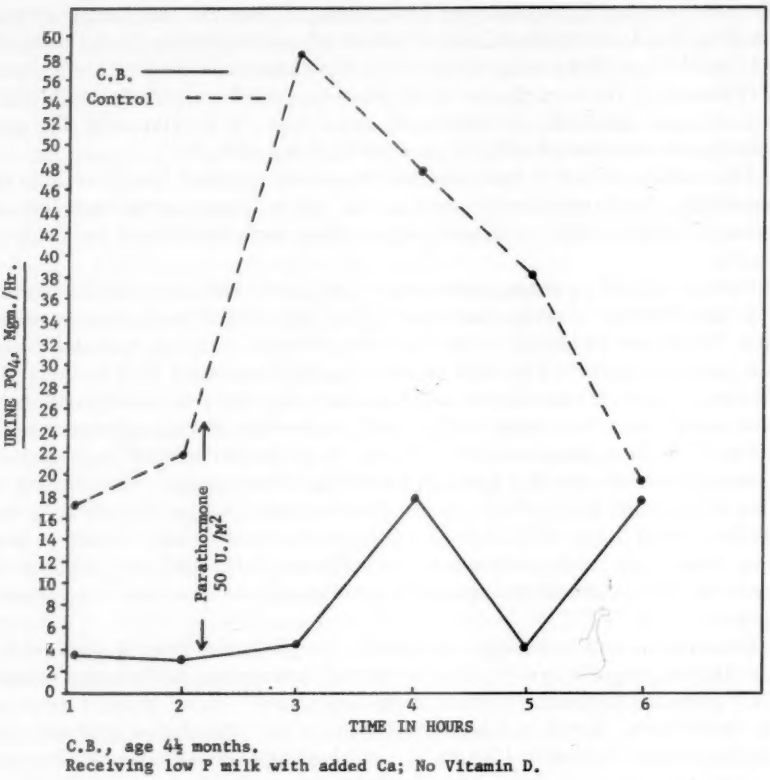
This patient is now 3 years old and is grossly retarded both mentally and physically. His extremities are spastic; he has no head control and does not speak. The EEG shows diffuse disorganization with no clearcut focal abnormality.

Case No. 2. D.B., a younger male sibling of Case 1, was hospitalized at the age of 5 days because of generalized convulsions beginning 2 hours prior to admission. There was no history of cerebral birth injury, cyanosis, or jaundice, and the child was afebrile. He had been receiving an evaporated milk formula (1:2 dilution). Physical examination was negative except for generalized convulsions and intermittent twitchings of face and extremities. Serum calcium was 2.1 mEq./L., with a phosphorus of 8.1 mEq./L. Urine Sulkowitch was negative. Lumbar puncture revealed normal spinal fluid. Blood sugar, serum electrolytes (Na, K, Cl, and CO_2), BUN, serum proteins and alkaline phosphatase were within normal limits. EEG showed a paroxysmal dysrhythmia. X-rays of skull, long bones and hands were normal. An Ellsworth-Howard test done on the tenth day of life showed an increase in urine phosphate in response to parathormone.

Convulsions and twitchings continued throughout the first 10 days of hospitalization, responding only for a few hours to intravenous injections of calcium. Oral calcium, aluminum hydroxide gel, and phenobarbital were started, and the patient was placed on a high Ca/P ratio milk. Convulsions gradually subsided as serum Ca rose to 4.5 mEq./L. and P dropped to 6.2 mEq./L. The urine Sulkowitch became positive. After 2 seizure-free weeks, for no apparent reason, the Sulkowitch again became negative, serum Ca dropped to 3.2 mEq./L., P rose to 7.3 mEq./L. and convulsions began again. These were controlled by increasing the amount of oral calcium given. Serum Ca rose to 4.0 mEq./L., and the urine Sulkowitch again became intermittently positive.

The patient was discharged from the hospital at the age of 9 weeks, on the above medications. He had no difficulty, except that the mother noted inspiratory stridor associated with feedings. He had no further convulsions during the next 9 months, although his serum Ca ranged between 3.6–4.1 mEq./L., and the P between 3.4–8.7 mEq./L.

At the age of 11 months, he developed a febrile illness and again began to have convulsions. During this hospital admission, the serum Ca ranged from 2.8 to 3.1 mEq./L., P from 6.0 to 7.0 mEq./L. Convulsions subsided after the first 3 days, when the patient became afebrile. However, because of persistently low Ca and high P, Vitamin D was started. Dosage has ranged from 50,000 to 200,000 units daily. The child is now 2 years old and has had no further con-

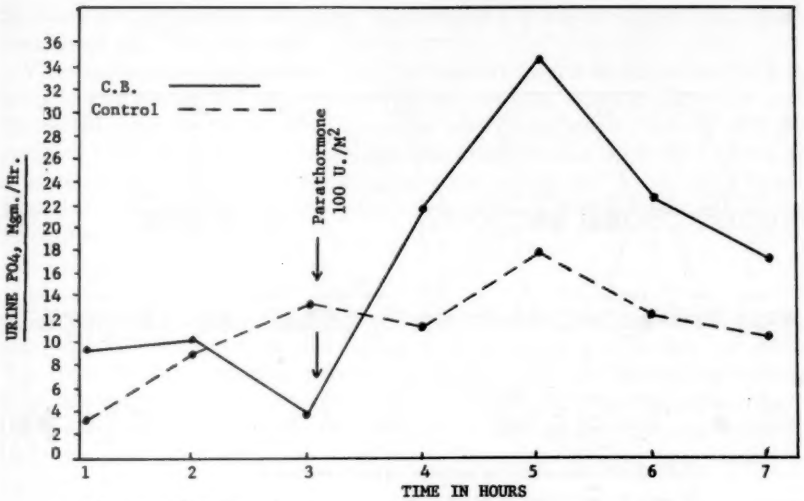


C.B., age 4½ months.
Receiving low P milk with added Ca; No Vitamin D.

FIG. 1

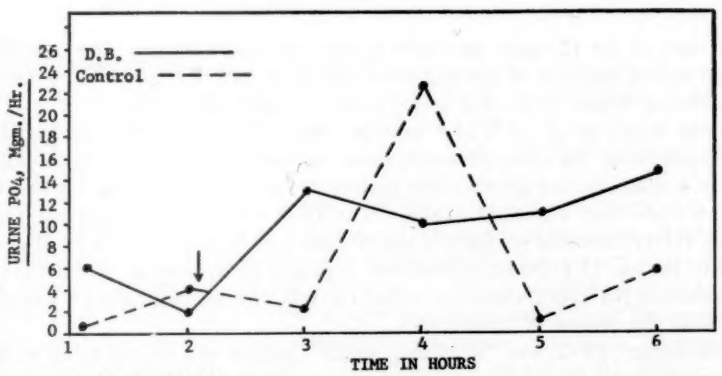
vulsions, although serum Ca and P remain slightly abnormal despite therapy. He is mentally normal and now has a normal EEG. He has no cataracts and has never had a monilial infection. Except for one brief episode of mild pyuria which readily responded to treatment, urinalyses have been consistently negative and the BUN remains normal.

The results of Ellsworth-Howard tests performed on Case 1 and Case 2 are shown in Figures 1, 2, and 3. Both patients, as compared with their controls, showed an adequate phosphate diuresis in response to parathormone. While a discussion of the intricacies of application and interpretation of the Ellsworth-Howard test is beyond the scope of this paper, it should be mentioned that this test, per se, is of little value in establishing a diagnosis of THP, and that even its use in distinguishing between THP and pseudohypoparathyrodism is open to some question (McGregor and Whitehead, 1954). However, the results presented here are compatible with the commonly described findings in THP as opposed to pseudohypoparathyroidism.



C.B., age 3 years.
Receiving Vitamin D 75,000 U./d., low P milk, added Ca.
(Vitamin D omitted on day of test).

FIG. 2



D.B., age 10 days.
No medication.

FIG. 3

THE PEDIGREE

The results of investigation of the family background of these cases are shown in Fig. 4. This information was obtained from the mother (IV-48) and maternal grandmother (III-24) of the two probands, and was independently corroborated by III-2. The hospital records of IV-8 and V-3 were examined, and V-3's physicians were consulted.

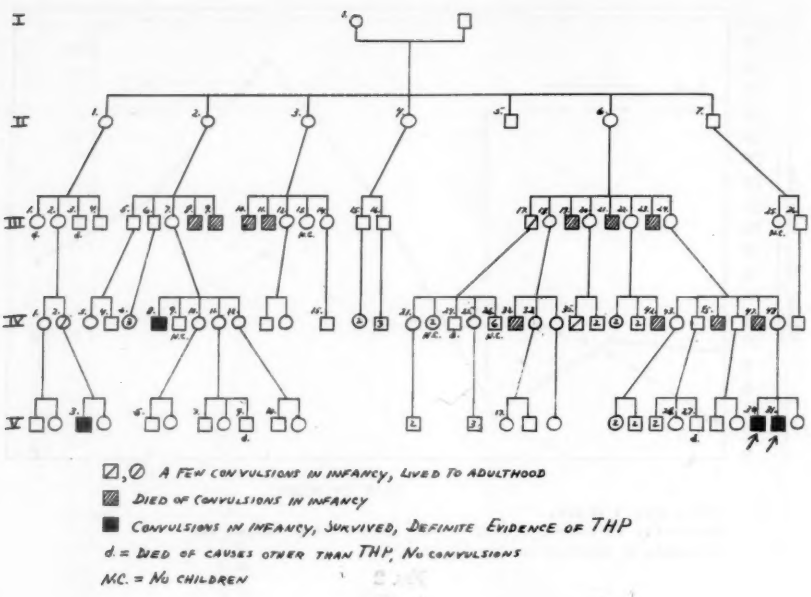


FIG. 4

In each of the 11 males who died of convulsions in infancy, the convulsions began before one year of age and were said to be afebrile. In seven of these the convulsions began between 2 and 8 weeks of age, and in the other four convulsions began at about 5 to 6 months. Seven of these babies died within a few weeks after the onset of convulsions, but four of them lived to the ages of 1½ to 4 years, having intermittent periods of convulsions throughout this time.

Two males had a few convulsions in infancy but lived to adulthood. One of these, III-17, has had no further convulsions and is said to be in good general health. He has 11 children, seven boys and four girls, none of whom have had convulsions. No information is at present available concerning the present status of IV-35, the second of these males.

One female, IV-2, had "quivering spells", lasting one or two minutes up to the age of about 18 months, and what was probably a generalized convulsion at the age of 5 years. Since then she has had no seizures and is said to be in good general health.

Males other than the probands, for whom there is known clinical evidence of THP:

IV-8 had convulsions at least as early as 9 months of age, associated with carpopedal spasm and laryngeal stridor. He was admitted to various hospitals in this city four times during his first two years of life, each time with typical tetany and convulsions which responded to calcium and Vitamin D. He is now

25 years old, somewhat mentally retarded, still has convulsions, and takes medication for "low calcium".

V-3 was diagnosed as probable THP at another local hospital, where he was admitted at the age of two weeks with convulsions, positive Chvostek and Trousseau signs, a low serum calcium and high phosphorus. These conditions persisted until about 2½ months of age, but finally responded to large doses of calcium and Vitamin D. This patient is now 3 years old, and is still under treatment. He has some degree of mental retardation and moderate spasticity of the extremities.

DISCUSSION

Examination of the pedigree of the two cases reported here indicates a probability that THP, at least in this family, is inherited as a sex-linked recessive trait. It is of interest that the maternal grandmother of the two probands, in a letter concerning her family history, rather neatly characterizes this mode of inheritance when she states: "I want to point out that no girls have had spasms. Only the girls' boys, and not all of them. No boys' children have had any spasms".

That the male relatives who died of convulsions in infancy suffered from THP cannot be proven, but seems likely. If this probability is accepted, then the pedigree fulfills the criteria of sex-linked recessive inheritance to the extent that only males are affected, the females being carriers and unaffected. None of the males related to a female carrier by a male parent or grandparent were affected.

Unfortunately, there is at present no clinical method of detecting the carrier state. Serum calcium and phosphorus were normal in both parents of the probands, and were also normal in the mothers of four other reported cases of neonatal THP (Buchs, 1955 and 1957; Rhyne, 1956), so these determinations are of no aid in detecting carriers.

To test the validity of the assumption that this pedigree represents sex-linked inheritance, computation was made of the expected incidence of affected and normal males on the maternal side of the probands' ancestry. There is no direct information concerning I-1. However, if this is sex-linked inheritance, then the mother (I-1) of the carrier women in Generation II must have carried the gene. With this assumption, calculations were made according to the method recommended by Steinberg (1959). This method is based on the theory that in a female known to carry the gene, the probability that she would pass it on to any particular son or daughter is 1/2, and the probability that this daughter, if she has the gene, would transmit it to any one son is again 1/2. So the probability that the first known carrier (I-1) would transmit the gene to any particular grandson or grand-daughter would be $1/2 \times 1/2$, or 1/4. To carry this a generation further, the probability of any particular great-grandson or great-granddaughter of I-1 receiving the gene is $1/2 \times 1/2 \times 1/2$, or 1/8. The probabilities are somewhat different for females having a direct blood relationship to the index

cases, as these women are known to carry the gene. Hence, for II-6, III-24, and IV-48, the probability of their transmitting the gene reverts to 1/2.

Results of computation made by this method are shown in table one. The deviation of observed from expected ratios is statistically not significant ($\chi_1^2 = .687$, P = .50–.30). The results, then, are consistent with the hypothesis that in this family THP is inherited as a sex-linked recessive trait.

The criterion of the inability of affected males to transmit the trait to or through their sons cannot be demonstrated, because the affected males who would now be old enough to have children died and did not reproduce. For the same reason, the invariable transmission of the carrier state to daughters of affected males cannot be shown.

The one possibly affected male (III-17) who survived and did reproduce did not transmit overt disease to any of his children or grandchildren. It is highly doubtful that III-17 actually had THP, as he is now in his fifties and is said to be in good health.

The possibility that this pedigree represents sex-limited inheritance of an autosomal gene, rather than sex-linked inheritance, cannot be excluded, as neither ability nor inability of affected males to transmit the gene to their sons can be demonstrated. However, to quote Roberts (1959), "Sex linkage in man is so common, and complete sex limitation almost certainly so rare, that

TABLE 1. EXPECTED AND OBSERVED NUMBERS OF AFFECTED AND NORMAL SONS OF I-1 AND OF EACH OF FEMALES RELATED TO I-1 VIA FEMALES

Females	Sons			
	Affected		Normal	
	Expected	Observed	Expected	Observed
I-1	1.00	0.0	1.00	2.0
II-1	.50	0.0	1.50	2.0
II-2	1.00	2.0	3.00	2.0
II-3	.50	2.0	1.50	0.0
II-4	.50	0.0	1.50	2.0
II-6	2.00	3.0	2.00	1.0
III-7	.25	1.0	1.75	1.0
III-12	.125	0.0	.875	1.0
III-14	.125	0.0	.875	1.0
III-18	.25	1.0	.75	0.0
III-20	.75	0.0	2.25	3.0
III-22	.75	1.0	2.25	2.0
III-24	2.00	2.0	2.00	2.0
IV-1	.063	0.0	.937	1.0
IV-2	.063	1.0	.937	0.0
IV-10	.063	0.0	.937	1.0
IV-11	.063	0.0	.937	1.0
IV-12	.063	0.0	.937	1.0
IV-33	.125	0.0	.875	1.0
IV-43	.50	0.0	1.50	2.0
Totals	10.69	13.0	28.31	26.0

Omitted: 2 Index cases (V-30 & V-31), and V-9 (died of unknown cause a few hours after birth).

the alternative explanation, while it cannot be disproved, must be considered somewhat fanciful".

On careful examination of the information furnished in available case reports of THP, it becomes apparent that there is more than one type of THP. The observed differences in clinical characteristics and genetic aspects suggest the following classification of types of THP:

I. Early-onset
   A. Sex-linked
   B. Non-sex-linked (congenital absence of parathyroid glands, associated with multiple congenital anomalies)

II. Late-onset
   A. Familial
      1. Addison's associated
      2. Non-Addison's associated
   B. Non-familial
      1. Addison's associated
      2. Non-Addison's associated.

Considering first the cases of early-onset THP, it should be noted that these constitute a minority of the total number of THP cases. Hypocalcemic convulsions due to idiopathic THP do not usually occur during the first year of life, most cases going several years before convulsions develop, and some cases never manifesting convulsions (Emmerson, 1941; Goldman, 1952). Table 2 lists the eight reported cases of idiopathic THP with convulsions beginning during the first year of life, plus the two cases reported here for the first time. Harrison (1956) mentions two other cases of neonatal THP, not included in the table, which apparently have not been formally reported. The sex of these two infants is not stated.

The predominance of males in this group is rather striking, in view of the fact that in Bronsky's review (1958) of 50 reported cases of all types of THP, 24

TABLE 2. REPORTED CASES OF TRUE IDIOPATHIC HYPOPARATHYROIDISM WITH CONVULSIONS BEGINNING DURING FIRST YEAR OF LIFE

Reference	Sex	Age at Onset Convulsions	Congenital Anomalies
Soper (1957)	M	Tetany in newborn period. Convulsions a 9 months.	None recorded
Bergstrand (1958)	M	"A few months less than 1 year"	None recorded
Buchs (1955)	M	7 days	None recorded
Buchs (1955)	M	7 days	None recorded
Buchs (1957)	M	3 weeks	None recorded
Forbes (1956)	M	5 days	None recorded
Rhyne (1956)	F	7 days	Bilateral congenital glaucoma
Symon (1959)	M	"A few weeks"	None recorded
This paper	M	5 days	None recorded
This paper	M	7 weeks	None recorded

Total Cases – 10; M – 9, F – 1

were male and 26 were female. If one were to include in table 2 the two cases of probable THP noted in the present pedigree (IV-8 and V-3), the total would be 12 cases, all but one of which occurred in males.

Of the cases in table 2, only those of Buchs and those from this hospital are recorded as being familial. Buchs' cases occurred in three male siblings, but he gives no family history except that the parents and a female sibling were normal. No extensive investigation of family history is mentioned in the other case reports, so the possibility that they were also familial, and perhaps sex-linked, cannot be excluded.

From the pedigree of the two patients reported in this paper, and from the marked predominance of males in the early-onset group of THP cases, the suggestion may be drawn that early-onset cases of THP represent a distinct and different type of the disease, inherited in a sex-linked manner. However, it does not necessarily follow that all cases of early-onset THP are of this type. Other types of THP might show an occasional case presenting convulsions earlier than is characteristic of those particular types.

Proof or disproof of the implication that early-onset THP is inherited in a sex-linked pattern would require, of course, a much more extensive investigation of the pedigrees of patients in this group. It would require, also, an explanation of the occurrence of neonatal THP in a female, as in the well-documented case reported by Rhyne (1956). This case was unusual also in that the child had congenital bilateral glaucoma. No family history was given in this report, other than the statement that the parents were normal and there were no siblings.

Rhyne's case, besides being the only female in the early-onset THP group, is also the only one of these cases in which any associated congenital anomaly is recorded. This case may perhaps belong to the small group described by Lobdell (1959), in which congenital absence of the parathyroid glands was associated with multiple other congenital anomalies. Lobdell reported a case of hypoparathyroidism in a male infant with hypocalcemic symptoms beginning during the first few days of life and death occurring at the age of 55 days despite therapy. At autopsy a careful search of the entire neck block revealed no parathyroid tissue, but the child was found to have a double aortic arch with a patent ductus arteriosus, absence of the thymus, and agenesis of the thyroid isthmus. Lobdell cites four similar cases, from the foreign literature, in which congenital absence of the parathyroids was associated with a variety of congenital anomalies such as acrania, microcephaly, aniridia, hypoplasia of the thymus, and partial thyroidal agenesis (Rössle, 1932 and 1938; Blaim and Lewicki, 1955). Two of these cases occurred in males, one in a female, and the sex of the fourth case is not recorded.

Another case similar to Lobdell's has recently been reported in the American literature by Farber and Vawter (1960). This was a female infant in whom findings of hypoparathyroidism were present from the age of 6 days, with death occurring at 12 days. At autopsy the only parathyroid tissue which could be found consisted of one very small group of cells, located high in the submucosa

of the posterior pharynx. There were multiple congenital anomalies, including absence of the gall bladder, a hypoplastic and ectopic thymus, hypoplasia of the jaw, hypoplasia of the lungs and tetralogy of Fallot.

It is possible that in addition to Rhyne's case, some of the other cases of early-onset THP recorded in table 2 also properly belong to this group, having congenital anomalies which were not apparent on external examination.

Another possible explanation for the appearance of neonatal THP in a female would be the expression of a sex-linked recessive trait in an individual who is only phenotypically a normal female.

Returning to the suggested classification of THP according to type, the late-onset type, which includes the majority of all cases, remains to be considered. This type may be tentatively divided into a familial variety, and a variety which is not apparently familial.

Familial cases of THP are unusual, there being recorded only 8 such cases in 3 families (table 3), excluding the three males in one family reported by Buchs and previously discussed as belonging to the early-onset group. In none of these three families was there any history of THP in previous generations. Of the eight familial cases of late-onset THP, six were of the type which is associated with Addision's disease, either in the patient or in other members of the family, and which is frequently also associated with monilial infections. The remaining two familial cases were not apparently associated with Addison's disease, and no moniliasis is described. It is entirely conceivable, however, that these last two cases might eventually belong to the Addison's-associated group, since in most instances of Addison's-associated THP the hypoparathyroidism precedes the Addison's, occasionally by several years.

The apparently non-familial group of late-onset THP cases, like the familial group, includes both Addison's-associated and non-Addison's-associated types. The nine reported cases of non-familial, Addison's-associated THP are shown in table 3. The remainder of the non-familial, late-onset group did not show signs of Addison's disease at the time of reporting, although some of them did manifest moniliasis. Some of these cases without Addison's disease, had they been followed long enough, might very likely have developed the disease. For example, the case of isolated THP originally reported by McQuarrie (1941) (table 3) was later mentioned by Papadatos (1954), in a report of another case, as having developed Addison's disease.

None of the early-onset cases of THP (table 2), familial or non-familial, had either Addison's disease or moniliasis at the time of reporting. It is possible, of course, that these conditions might later appear in any of them.

The mechanisms responsible for a deficiency of parathyroid hormone in any type of THP are unknown. A search of the literature for autopsied cases of THP reveals only seven such cases (table 4), aside from those previously mentioned as being associated with multiple congenital anomalies. All of these seven cases were of the late-onset, non-familial type, and five of them were associated with Addison's disease. In six cases no parathyroid tissue was found, but in one case parathyroid remnants were discovered. In view of the relatively late

TABLE 3. FAMILIAL, LATE-ONSET THP AND ADDISON'S-ASSOCIATED THP

Reference	Sex	Familial	Addison's	Monili-asis	Age at Onset THP
Sutphin (1943)	M	+	+	+	10 yrs.
	F	+	+	+	6 yrs.
		One Family			
	F	+	+	+	12 yrs.
Goldman (1952)	M	+	+ (Family history)	−	10 yrs.
	M	+	+	−	12 yrs.
		One Family	(Family history)		
	M	+	+ (Family history)	−	12 yrs.
Forbes (1956)	M	+	−	−	3½ yrs.
		One Family			
	F	+	−	−	2½ yrs.
McQuarrie (1941)	M	−	+	−	?5½ yrs.
Whitaker (1956)	M	−	+	+	15½ yrs.
Malloy (1958)	M	−	+	+	?6 yrs.
Forbes (1956)	F	−	+	−	?3 yrs.
Forbes (1956)	F	−	+	−	4½ yrs.
Perlmutter (1956)	F	−	+	−	10 yrs.
Leifer (1953)	M	−	+	−	?3 yrs.
Leonard (1946)	F	−	+	−	?3 yrs.
Papadatos (1954)	M	−	+	−	?10 yrs.

TABLE 4. AUTOPSIED CASES OF THP

Reference	Sex	Age at Onset THP	Addison's	Familial	Parathyroid Glands
Perlmutter (1956)	F	10 yrs.	+	−	None found
Whitaker (1956)	M	15½ yrs.	+	−	None found
Leonard (1946)	F	?3 yrs.	+	−	None found
Forbes (1956)	F	?3 yrs.	+	−	None found
Papadatos* (1954)	M	?5½ yrs.	+	−	None found
Cantarow (1939)	F	3½ yrs.	−	−	None found
Drake (1939)	M	4 yrs.	−	−	Parenchyma replaced by fat cells in all 4 glands.

* Papadotos refers to the follow-up on a case previously reported by McQuarrie (1941)

onset of hypocalcemic symptoms, it is difficult to imagine how the parathyroid glands could have been absent from birth in these cases. It would seem more likely that the development of THP was the result of later damage to the glands, either by exogenous or endogenous factors.

In the cases of early-onset THP (table 2) it might be postulated that the

parathyroid glands are congenitally absent. Another theory might be that of an inborn enzymatic defect interfering with the normal metabolism of parathyroid hormone (or hormones), similar to the enzymatic blocks described in congenital adrenal hyperplasia (Bongiovanni, 1958; Eberlein, 1958) and in goitrous cretinism (Stanbury, 1957).

No information is available regarding post-mortem findings in the early-onset, sex-linked type of THP. Theoretically, however, an enzymatic defect such as postulated could result either in damage to the glands with subsequent atrophy, or in hypertrophy due to an attempt at compensation for inadequate function. Or, compensatory hypertrophy might precede ultimate atrophy. Pursuing the theory of an enzymatic block still further, differences in degree of block might account for some of the differences in age at onset of symptoms and length of survival exhibited by cases of early-onset THP.

It is hoped that the report presented here will arouse interest in more extensive investigation of pedigreees of patients with THP. It is hoped, also, that the classification of THP presented here, although it is obviously tentative, will stimulate authors of new case reports to evaluate their findings in relation to other information in the literature, rather than simply as individual cases. From this approach may come some clarification of the present vast confusion concerning this disease.

SUMMARY

Two cases of neonatal true idiopathic hypoparathyroidism ocuring in male siblings are reported. A pedigree is presented which indicates sex-linked inheritance of THP in this family, and the suggestion is made that the majority of cases of early-onset THP may be inherited in this same pattern.

A distinction is drawn between this sex-linked, early-onset type of THP, and a non-sex-linked type of later-onset. The latter type is divided, on the basis of clinical observations, into familial and apparently non-familial varieties, each of which is further subdivided into those associated with Addison's disease and those which are not apparently Addison's-associated. What portion of the non-sex-linked, late-onset group is genetically determined is at present uncertain.

More thorough investigation of the ancestry of cases of THP reported in the future will serve to clarify what is now merely an implied division of THP into these different types.

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Distribution of Haptoglobin, Transferrin, and Hemoglobin Types Among Indians of Southern Mexico and Guatemala

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HAPTOGLOBINS are part of the α_2-glycoprotein fraction of human plasma and were first detected by Polonovski and Jayle (1938) because of the ability of haptoglobin to complex with hemoglobin. Smithies (1955a, 1955b) demonstrated the existence of polymorphism in these proteins, and, with Walker, worked out the mode of heredity (Smithies and Walker, 1955, 1956). Briefly stated, there are three major haptoglobin phenotypes under the control of a two-allele system. Genotype Hp^1/Hp^1 produces haptoglobin type 1-1 (Hp 1-1); Hp^2/Hp^1 produces Hp 2-1; and Hp^2/Hp^2 produces Hp 2-2. The techniques for distinguishing these types by means of starch gel electrophoresis have been described by Smithies (1955b).

Several other types have been reported recently (Galatius-Jensen, 1958; Connell and Smithies, 1959). Only one of these, the Hp 2-1 (modified) of Connell and Smithies, has been found to occur frequently. No complete genetic studies have been published on this type. It can be confused with Hp 2-1 and in some studies may contribute to the 2-1 frequency. Other new types appear to be so rare as not to constitute a serious source of error in estimating gene frequencies.

A lack of detectable haptoglobin has been observed in some individuals by a number of investigators (e.g. Allison, Blumberg, and ap Rees, 1958; Galatius-Jensen, 1958; Giblett, 1959; Sutton et al., 1959). This condition can arise both as a result of genetic and of environmental factors (Laurell and Nyman, 1957; Allison and ap Rees, 1957). The inherited form does not appear to be controlled by alleles at the usual haptoglobin locus (Galatius-Jensen, 1958; Sutton et al., 1959). Recent studies suggest that some ahaptoglobinemics actually possess small amounts of normal haptoglobin (Sutton et al., 1959).

A comparison of *Hp* frequencies in different populations was first made by Sutton et al., (1956), who found a higher frequency of the Hp^1 allele among African Negroes than among Caucasians. A number of population surveys have since been reported, and it is apparent that *Hp* frequencies vary considerably among groups of different racial origin. It is interesting, nevertheless, to note that both common alleles have been found in every population thus far examined.

The same starch gel electrophoresis techniques which have proved so useful

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in separating haptoglobins can also be used to reveal inherited variations in transferrin of human plasma (Smithies, 1957; Horsfall and Smithies, 1958). Most individuals possess a single type of transferrin, designated Tf C. Others possess two types of transferrin, Tf C and Tf D. A few individuals possess only Tf D. Analysis of pedigrees suggests that the genotype of Tf C individuals is Tf^C/Tf^C, that of Tf D individuals is Tf^D/Tf^D, and that of Tf CD individuals is Tf^C/Tf^D. Several additional transferrin types have been discovered since these first reports (Smithies, 1958; Harris, Robson, and Siniscalco, 1958; Smithies and Hiller, 1959; Giblett, Hickman, and Smithies, 1959). Those transferrins which migrate toward the anode (pH 8.5) faster than the common Tf C are designated Tf B with numerical subscripts for further differentiation (Tf B_0, Tf B_1, etc.). Transferrins which migrate slower than Tf C are designated Tf D_0, Tf D_1, etc. The transferrins for which family data are available appear to be inherited as the result of single allelic differences. Unfortunately there are no families reported in which more than two types of transferrin are present; hence, it is impossible to be sure whether all the transferrins result from variation at a single genetic locus.

The frequency of transferrin types also varies among populations, Tf D having been found only among Negroes and Australian aborigines, while Tf B has been observed only among whites. However, other than the common Tf C, Tf D is the only transferrin which occurs frequently. These results are based on very limited population surveys.

In this paper we shall report the frequencies of haptoglobin, transferrin, and hemoglobin types among certain Indian tribes of Southern Mexico and Guatemala. Results of studies of hereditary blood factors among these Indians were presented in a previous publication (Matson and Swanson, 1959).

EXPERIMENTAL METHODS

The subjects of this study were putatively full blood Indians, although the possibility that a few genes have been introduced from other populations must be entertained. Venous blood samples were collected in citrate and shipped by air to Minneapolis, where the plasma was separated and frozen. The location of the tribes from which samples were collected is indicated in Fig 1. Additional details are given in Matson and Swanson (1959).

The frozen plasma samples were shipped by air to Ann Arbor for ascertainment of the haptoglobin and transferrin types. The vertical starch gel method of Smithies (1959) was used. As usual the gel was sliced into two parts, one of which was stained with amidoschwarz 10-B to reveal proteins (Smithies, 1955b), the other was stained for hemoglobin (peroxidase activity) with *o*-dianisidine (Owen, Silberman, and Got, 1958). All samples were tested both without and with added cyanmethemoglobin, except those few which were already somewhat hemolyzed, to observe the haptoglobins both free and complexed with hemoglobin. In general, the haptoglobin bands were among the strongest encountered in this laboratory, and only a few samples required more than routine handling for classification.

Five samples were encountered in which no haptoglobin could be detected by

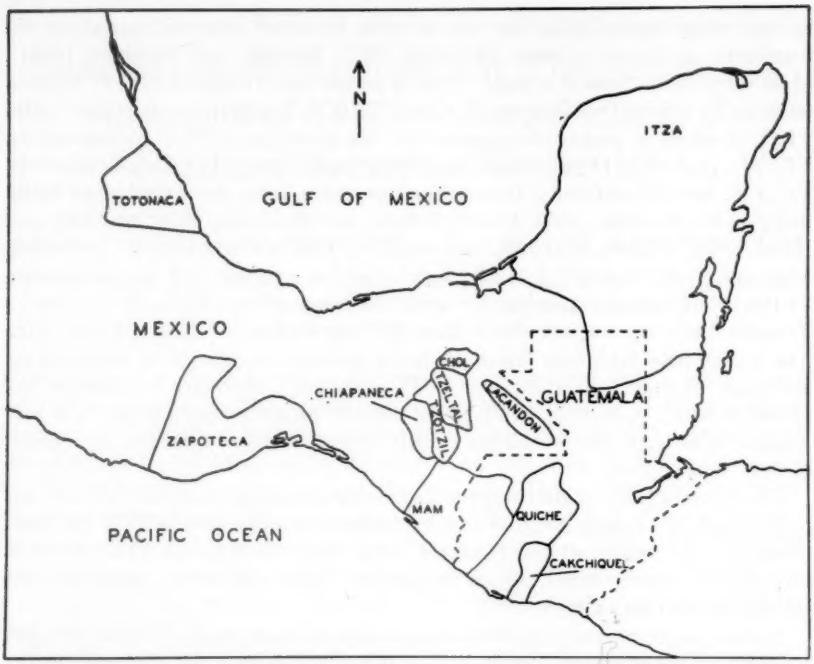

FIG. 1. Location of Indian tribes from whom blood samples were collected. Samples from the Itza were collected only in the northern part of the Yucatan Peninsula. Based in part on Kroeber (1939) and on Morley (1946).

electrophoresis. The quantitative haptoglobin assay of Connell and Smithies (1959) showed that these samples had about one-tenth the normal haptoglobin activity. Thus some haptoglobin was present, but the individuals were classified as "ahaptoglobinemic" (Hp O) since they could not be assigned to a conventional type. This is in accordance with previously reported observations on ahaptoglobinemia (Sutton et al., 1959).

For the determination of hemoglobin types, erythrocytes were washed three times with isotonic saline solution and duplicate samples of hemolysates were prepared by a modified technique of Drabkin (1946). These solutions were frozen and then sent to Dr. R. W. Koucky, Fairview Hospital Laboratory, Minneapolis, who determined hemoglobin types by a method using the Spinco paper electrophoresis system. Duplicate hemolysates were sent to Dr. Wolf W. Zuelzer and Mr. Abner R. Robinson, Child Research Center Laboratories, Detroit, Michigan, where they were tested by the method of agar electrophoresis at pH 6.5, citric acid buffer (Robinson et al., 1957) and by paper electrophoresis at pH 8.6, veronal buffer, ionic strength 0.06 (Robinson et al., 1957).

RESULTS

The results of the haptoglobin and transferrin determinations are given in table 1. Only the Lacandons deviate significantly in their haptoglobin types

TABLE 1. DISTRIBUTION OF HAPTOGLOBIN AND TRANSFERRIN TYPES AMONG INDIANS OF SOUTHERN MEXICO AND GUATEMALA

Tribe	Number	Haptoglobin Type						Transferrin Type		
		0	1-1	2-1	2-2	2-1 (mod.)	Hp^1 frequency	C	BC	CD
Maya:										
Lacandon	31	3	24	4	0	0	.93	31	0	0
Itza	86	1	21	45	17	2	.52	84	0	2
Mam	27	0	8	11	8	0	.50	27	0	0
Quiche	94	0	36	46	12	0	.63	92	1	1
Chol	16	0	9	4	3	0	.69	16	0	0
Tzotzil	88	0	31	44	13	0	.60	87	1	0
Tzeltal	97	0	31	54	12	0	.60	97	0	0
Cakchiquel	10	1	4	4	1	0	.67	10	0	0
Non-Maya:										
Zapoteca	80	0	22	40	17	1	.53	78	2	0
Chiapaneca	47	0	21	20	5	1	.67	45	0	2
Totonaca	45	0	14	23	8	0	.57	45	0	0
Mestizo	17	0	5	8	4	0	.53	17	0	0
Totals	638	5	226	303	100	4		629	4	5

from the other tribes. No other heterogeneity could be detected, either within the Maya and non-Maya groups or between Maya and non-Maya groups. The Hp^1 frequency for the Maya (excluding Lacandons) was found to be 0.59. For the non-Maya it was 0.57. If the two groups are pooled, the overall average is 0.58.

In accordance with other studies, no association of sex with haptoglobin type could be detected. Similarly, comparison of the median age of each haptoglobin type failed to reveal any significant differences. The proportion of the three types agreed very well with prediction based on the Hardy-Weinberg Law.

The frequency of transferrins other than Tf C is too low to permit statistical analysis of the results.

Two hundred seventy four samples were tested for hemoglobin types. Of these, 227 were Mayan consisting of 34 Lacandon, 13 Chol, 57 Tzeltal, 6 Mam, 39 Quiche, 67 Itza and 11 Tzotzil, and 47 were non-Mayan consisting of 6 Chiapaneco, 7 Mextizo, 18 Totonaco and 16 Zapoteca. None showed evidence of abnormal homoglobin by either of the three techniques employed, all being the normal adult A type. One sample on paper at pH 8.6 exhibited an increased hemoglobin A_2 component. However, the amount of this sample was insufficient to attempt a quantitative determination by the starch block technique of Kunkel (1955). Fetal hemoglobin as estimated from the agar techniques was normal in all the samples.

DISCUSSION

A number of population surveys for haptoglobin types have been carried out. A summary of some of these is presented in table 2. The results from Central American Indians agree very well with the previously reported results from Apaches, although both these groups have a higher Hp^1 frequency than do the Athabascans of Alaska. This is particularly interesting since both the Apaches

TABLE 2. DISTRIBUTION OF HAPTOGLOBINS IN VARIOUS POPULATIONS

(Hp O individuals were subtracted from the total population before computation of Hp^1.)

Population	N	Hp^1	Hp O	Reference
Caucasian:				
U. S. White				
Ann Arbor	68	.43	0	Sutton et al. (1959)
Seattle	350	.38	.003	Giblett (1960)
Canadian	49	.46	0	Smithies (1955b)
British	180	.43	.005	Harris et al. (1959)
British	218	.39	.03	Allison et al. (1958)
Australian White	100	.43*	0	Budtz-Olsen (1958)
Australian White	323	.38	.003	Kirk et al. (1960)
Swedish	160	.41	0	Nyman (1958)
Swedish	220	.44	0	Beckman (1959)
Norwegian	1000	.36	0	Fleischer and Lundevall (1958)
Danish	2046	.40	0	Galatius-Jensen (1958b)
Finnish	891	.36	.002	Mäkelä et al. (1959)
Swedish Lapps	329	.32	.02	Beckman and Mellbin (1959)
Greenlanders	74	.30	0	Galatius-Jensen (1960)
Bavarians	273	.46	0	Baitsch and Meier (1959)
Swiss	920	.40	0	Bütler et al. (1959)
French	406	.40	0	Moullec and Fine (1959)
Italian				
Berra	120	.41	.01	Harris et al. (1959)
Cologna	208	.35	0	Harris et al. (1959)
Naples	93	.34	0	Harris et al. (1959)
Sardinia	147	.37	0	Harris et al. (1959)
Sicily	107	.40	0	Harris et al. (1959)
Spanish Basques	107	.37	.01	Allison et al. (1958)
Iranian	34	.25	0	Harris et al. (1959)
Indian	74	.18	0	Sutton et al. (1959)
Indian	33	.17	0	Giblett (1960)
Indian	219	.09	.02	Kirk et al. (1960)
Venezuelans ("hybrid")	208	.55	0	Gallango de Rodríguez and Arends (1959)
Mongolian:				
Orientals (Seattle, mostly Japanese)	242	.25	0	Giblett (1960)
Japanese	349	.24	.01	Matsunaga and Murai (1960)
Japanese	488	.27		Yamaguchi et al. (1959)
Japanese (U.S.A.)	23	.30	0	Harris et al. (1959)
Malays	236	.24	.01	Kirk et al. (1960)
Chinese	167	.28	.01	Kirk et al. (1960)
Alaskan:				
Eskimo	167	.33	.005	Giblett and Motulsky (1960)
Eskimo	418	.30	0	Blumberg et al. (1959)
Anaktuvuk	57	.52	.04	Blumberg et al. (1959)
North Athabascan	202	.42	.01	Blumberg et al. (1959)
Tlingit	82	.44	.02	Blumberg et al. (1959)
Apaches	98	.59	0	Sutton et al. (1959)
Central American				
Non-Maya	170	.57	.01	This report.
Maya (less Lacandon)	414	.59	0	This report.
Lacandon	31	.93	.10	This report.
Peruvian Indians	173	.73	0	Giblett and Best (1960)
Oceanic:				
Borneo	22	.50	0	Harris et al. (1959)
Micronesian (Marshall Islands)	52	.58	0	Blumberg (1959)
Tongans	200	.60	0	Douglas and Staveley (1960)

TABLE 2—*Continued*

Population	N	Hp^1	Hp O	Reference
Negroid:				
American Negro				
Ann Arbor	43	.59	.10	Sutton et al. (1959)
Seattle	760	.54	.04	Giblett (1960)
California	51	.61	.02	Harris et al. (1959)
Nigeria:				
Yoruba	99	.87	.32	Allison et al. (1958)
Yoruba	30	.72	.23	Harris et al. (1959)
Habe	120	.60	.27	Barnicot et al. (1960)
Fulani	111	.76	.37	Barnicot et al. (1960)
Liberia, Ivory Coast	614	.72		Sutton et al. (1959)
Gambia	157	.70	.40	Harris et al. (1959)
Ibo	70	.49	.48	Harris et al. (1959)
Congo				
Metropolitan	186	.60	.05	Motulsky and Giblett (1960)
Non-Metropolitan	468	.57	.21	Motulsky and Giblett (1960)
Pygmy	125	.40	.31	Motulsky and Giblett (1960)
Cape Colored	88	.47	0	Barnicot et al. (1959)
Hottentot	59	.51	0	Barnicot et al. (1959)
Zulu	116	.53	.03	Barnicot et al. (1959)
South African (Xhosa and Msutu)	315	.55	.05	Giblett and Zoutendyk (1960)
Bushmen	113	.29	.02	Barnicot et al. (1959)
Australian aborigines				
Central Australia	100	.63*	0	Budtz-Olsen (1958)
North Queensland	123	.46*	0	Budtz-Olsen (1958)
Western Desert	133	.17	.01	Kirk (1959)

* Based on paper electrophoresis only.

and the Athabascans are derived from the same linguistic stock. One must therefore invoke either selection or genetic drift to explain the differences which have arisen. In arriving at an answer, it may be noted that the Hp^1 frequency increases regularly from a low value of 0.22 in Japan to intermediate values in Alaska to fairly high values in Mexico and Peru. This suggests that the frequencies are not entirely a matter of chance and supports the idea that selection is important in maintaining a given gene frequency. The nature of the selective factors has not been elucidated.

Of the tribes studied in Central America, only the Lacandons show a haptoglobin distribution distinct from the other groups. There are fewer than 200 individuals of this tribal affiliation still in existence. They have remained quite isolated from the surrounding groups and reside in small clans in remote parts of a tropical rain forest. Because of their isolation, they have become very inbred, with several brother-sister matings having been recorded. To what extent their deviant haptoglobin frequencies are the result of genetic drift or to unusual selective factors in their environment cannot be ascertained at present. In support of the drift hypothesis is the fact that the Rh gene frequencies are somewhat deviant for the Lacandons. For example, R^1 has a frequency of .35 and R^2 of .65,

compared to .52 and .41, respectively, for the total Maya sample (Matson and Swanson, 1959). It is interesting to note that very high Hp^1 frequency associated with relatively frequent ahaptoglobinemia has also been reported in West Africa (table 2).

There are few reports available on the distribution of transferrins among populations other than European and Negro (Horsfall and Smithies, 1958; Smithies and Hiller, 1959; Giblett, Hickman, and Smithies, 1959). The Central American Indians in this study have been found to resemble the Caucasions in possessing almost exclusively Tf C, with a very low frequency of Tf BC.

The question of racial purity always arises in population surveys of this sort. In the present case the answer can perhaps best be obtained from examination of the results of the erythrocyte typing (Matson and Swanson, 1959). The finding of a few individuals of blood group A_2 and K^+ suggests that non-Indian genes have been introduced into at least some of these populations. The general concordance with blood group frequencies in other Indian populations is evidence that such admixture cannot be appreciably greater than 5 percent and may be less in some tribes.

It is interesting to consider some of the rarer haptoglobin and transferrin phenotypes in relationship to the "introduced" red cell antigens. For example, Hp 2-1 (mod.) has been previously found almost exclusively in persons of Negro ancestry, in whom it occurs with a frequency of 10 percent (Giblett, 1959). Of the four examples reported in this paper, two were found in the Itza and one each in the Zapotecas and Chiapanecas. Transferrin type CD, which also occurs primarily among Negroes, is found in the Itza (2 cases), the Quiche (1 case), and the Chiapanecas (2 cases). Tf BC is rare in all populations studied, so that the significance of a few individuals of this type cannot be evaluated. Examination of the red cell antigen data reveals that the Itza, the Chiapanecas, and the Zapotecas are the tribes most deviant in those antigens which might indicate admixture. One may therefore speculate that the gene which gives rise to Hp 2-1 (mod.) and the Tf^D gene occur in these Indians as a result of having been introduced from non-Indian groups. Since they are essentially Negroid characteristics, the introduction would presumably have been from Negro ancestors. One would expect some abnormal hemoglobins to occur among these Indians if there has been significant introduction of genes from Negro populations. The frequency of abnormal hemoglobins would be very low, however, and with the limited number of individuals tested in this survey, it is possible that the failure to find a single case of abnormal hemoglobin is a function of sampling.

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SUMMARY

Starch gel electrophoresis of haptoglobins and transferrins have been carried out on 449 Maya and 189 non-Maya Indians, and hemoglobin types have been studied on 227 Maya and 47 non-Maya Indians of Southern Mexico and Guatemala. Excluding 31 Lacandons, the Hp^1 frequency averaged 0.58, with no demonstrable heterogeneity. There was no association of haptoglobin phenotype with age or sex. The Hp^1 frequency of the Lacandons was 0.93; in addition, three of the Lacandons were ahaptoglobinemic.

Of the 638 individuals, four were of transferrin type BC and five were Tf CD, the remainder being Tf C.

The association of Hp 2-1 (mod.), Tf CD, Kell and A_2 antigens in certain tribes suggests that there has been some introduction of genes from other populations, possibly Negroid. This admixture would appear not to exceed five percent.

All bloods tested for hemoglobin appeared to be normal adult A type. None showed evidence of abnormal hemoglobin by either paper or agar electrophoresis methods. Fetal hemoglobin as estimated from the agar technique was normal in all the samples.

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The Mutational Load Due to Detrimental Genes in Man*

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MANY STUDIES HAVE DEMONSTRATED that children of consanguineous marriages suffer higher average mortality and morbidity than non-inbred children. Morton, Crow, and Muller (1956) made this fact the basis of an estimate of the total *genetic load* that would be expressed as premature mortality if an average gamete were doubled to produce a complete homozygote. Then, by identifying this load with mutational damage, they estimated the total mutation rate.

Lethal equivalents.—In order to calculate the genetic load expressed as mortality, they required only the assumption that the recessive genes contributing to mortality are to a first approximation independent in action, "nonsynergistic" in the sense used by Muller (1950), so that the probability of survival follows first order kinetics,

$$S = e^{-(A+BF)},$$

where A is the number of *lethal equivalents* expressed in a randomly mating population, B is the number of *lethal equivalents* brought to expression by inbreeding, and F is Wright's coefficient of inbreeding. The genetic load lies between B and A + B lethal equivalents per gamete (Morton, Crow, and Muller, 1956). The assumption of independent gene action seems justified by its simplicity and consistency with available data on inbreeding effects (admittedly scanty), and by the fact that interactions which may occur at high levels of inbreeding do not affect estimates made from the low levels which can be observed in man.

Thus estimation of the genetic load presents no difficulties. However, identification of this load with mutational damage is not so easy. If detrimental genes are maintained by mutation pressure acting against selection, then the situation may be represented as follows for a single locus:

Genotype	*GG*	*GG'*	*G'G'*
Frequency	$q^2(1 - F) + qF$	$2q(1 - q)(1 - F)$	$(1 - q)^2(1 - F) + (1 - q)F$
Relative fitness	$1 - s$	$1 - hs$	1

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Expressed genetic load = $s[q^2(1 - F) + qF] + hs[2q(1 - q)(1 - F)]$ per zygote.

Total genetic load = qs per gamete.

Here q is the frequency of the mutant gene *G*, s is the probability of death due to homozygosis for *G*, and h is a measure of dominance, being 0 if *G* is completely recessive and 1 if *G* causes the same probability of death in a heterozygote as in a homozygote. By summing the expressed loads over all loci and collecting the terms which do or do not involve F, we see that the expressed load is A + BF lethal equivalents per zygote and that the total load $\sum$ qs lies between B and A + B lethal equivalents per gamete, where

$$A = \sum x + \sum q^2 s + 2\sum q\,(1 - q)sh,$$

$$B = \sum qs - \sum q^2 s - 2\sum q\,(1 - q)sh,$$

and x is the load due to any particular environmental cause of death (Morton, Crow, and Muller, 1956).

The principal alternative to this model is a segregation load due to heterozygote advantage, with alleles G^i, G^j and gene frequencies q_i, q_j:

Genotype	G^iG^i	G^iG^j	G^jG^j
Frequency	$q_i^2(1 - F) + q_iF$	$2q_iq_j(1 - F)$	$q_j^2(1 - F) + q_jF$
Relative fitness	$1 - s_i$	1	$1 - s_j$

Expressed genetic load = $s_i[q_i^2(1 - F) + q_iF] + s_j[q_j^2(1 - F) + q_jF]$ per zygote.

Total genetic load = $q_is_i + q_js_j$ per gamete.

Again we find that the expressed load is A + BF lethal equivalents per zygote and the total load $\sum$ qs lies between B and A + B lethal equivalents per gamete, where

$$A = \sum x + \sum q^2 s,$$

$$B = \sum qs - \sum q^2 s,$$

and the summation, as before, is over all genotypes and causes of death (Crow, 1958).

These two models have very different consequences, since the relation between mutation rate and mutation load can be predicted, but the segregation load does not depend on mutation pressure and responds unpredictably to changes in mutation rate. It is therefore an important problem in population genetics to determine whether the genetic load estimated from inbreeding effects on morbidity is largely a mutational or segregational load.

Morton, Crow, and Muller (1956) attempted to distinguish between the two kinds of loads from the theorem, published in extenso by Crow (1958), that for the segregation load the ratio of B to A is strictly less than the mean number of mutually heterotic alleles per locus. In their data, B was about 17 times the

value of A. If environmental causes of death could be eliminated from A, the ratio B/A would certainly be greater than 17. Since this seems like an excessive number of mutually heterotic alleles to postulate at each locus, they concluded that the genetic load which they had estimated was largely a mutational load.

However, it should be noted that the argument based on B/A does not hold unless we measure total fitness. For example, if one allele were eliminated by mortality and another allele by sterility, we could not obtain from mortality data alone a valid estimate of the number of mutually heterotic alleles.

Detrimental equivalents.—The next step after publication of this paper was to extend the theory to detrimental traits not measured as mortality. Let t be the frequency with which a homozygote expresses the trait in question, th be the trait frequency in the heterozygote, q be the gene frequency, and x be the probability of a particular type of phenocopy (an environmentally induced modification which simulates the genetic trait). They by a straightforward application of the argument for lethal equivalents, the frequency of persons with the trait is

$$P = 1 - e^{-(A+BF)},$$

where

$$A = \sum x + \sum q^2t + 2\sum q\,(1 - q)th,$$
$$B = \sum qt - \sum q^2t - 2\sum q\,(1 - q)th,$$

on the mutational load model, and

$$A = \sum x + \sum q^2t,$$
$$B \doteq \sum qt - \sum q^2t,$$

on the segregational load model. The expressed load is A + BF detrimental equivalents per zygote, and the total load $\sum$ qt lies between B and A + B detrimental equivalents per gamete.

Several published bodies of data lend themselves to this kind of analysis (table 1). The most recent material on major congenital malformations was collected by the Atomic Bomb Casualty Commission (Schull, 1958). Consanguinity was recorded through a nearly complete registration of pregnant women in Hiroshima, Nagasaki, and Kure during 1948–1954, followed by home visits shortly after birth.

Another study (Sutter, 1958) was based on Catholic marriage dispensations issued during 1919–1925 in three French departments. The consanguineous families were visited by the authors, who took notes on conspicuous abnormalities. The same information was obtained from town clerks for a control sample of unrelated parents married during the same period and selected without regard to medical history. The dangers in this sampling scheme are obvious, but the data satisfy tests of internal consistency.

Slatis et al. (1958) studied consanguineous marriages (nearly all between first cousins) in the Chicago region, ascertained through Catholic marriage dispen-

TABLE 1. MORBIDITY IN CONSANGUINEOUS MARRIAGES

(Data given as defective/total, with proportion of defectives below)

Source	First cousins (F = 1/16)	1-1/2 cousins (F = 1/32)	Second cousins (F = 1/64)	Other relationship (F = .1134)	Not related (F = 0)
Schull (1958): major congenital malformation	48/2846	8/838	13/1161	—	651/63,796
	.017	.010	.011		.010
Sutter (1958): abnormality	169/1044	34/259	73/953	—	176/4094
	.162	.131	.077		.043
Slatis et al. (1958): abnormality	31/192	—	—	—	16/163
	.161				.098
Böök (1957): genetic abnormality	41/218	—	—	—	13/165
	.188				.079
mental deficiency	7/128	—	—	—	81/6340
	.055				.013
Bemiss (1858): abnormality	190/525	—	102/561	243/447	—
	.362		.182	.544	

sations issued during 1936–1956. These were compared with a control sample of the families of married sibs, preference being given to a sister of the wife.

Böök (1957) examined all marriages between first cousins in three north-Swedish parishes. For each cousin family another family living in the nearest house or farm was selected as a control. He chose for analysis abnormalities commonly considered to have a major genetic component, not necessarily recessive. Population data were available for mental deficiency (corresponding approximately to an I.Q. less than 70). Consanguineous marriages between normal or gifted parents are compared with the population incidence in Table 1.

As a final sample, we may consider data collected by Bemiss (1858) a century ago through correspondence with physicians and recently reclassified by Sutter and Tabah (1951). This material probably gives an upper estimate of the effect of inbreeding, not only because of unintentional bias in selection of families, but also because the population lived in a more rigorous environment and abnormality was broadly defined to include infectious diseases. Bias in selection of families can be minimized, although probably not eliminated, by considering only degrees of consanguinity, all of which were ascertained by the same procedures, and omitting the unrelated parents who were ascertained differently.

Estimates of A and B were obtained from the regression on F of the natural logarithm of the frequency of normals, weighted by the amount of information $n(1 - P)/P$, where n is the number of observations and P is the expected fraction of abnormals. The weights were obtained by iteration, starting with the observed value of P as a trial value. Samples with more than two values of F provide a test of internal consistency of the data. This test is applicable to the samples of Bemiss, Sutter, and Schull, all of which are in agreement with the model.

The estimates of A and B are given in table 2. The ratio B/A tends to be higher in these studies than in comparable ones on mortality, suggesting a relatively

TABLE 2. ESTIMATES OF A AND B

	A	B	B/A	σ_B	χ^2
Schull (1958): major congenital malformation	.0102	0.093	9.1	0.036	1.26
Sutter (1958): abnormality	.0443	2.213	49.9	0.211	1.82
Slatis et al. (1958): abnormality	.1033	1.164	11.3	0.654	—
Böök (1957):					
genetic abnormality	.0821	2.020	24.6	0.636	—
mental deficiency	.0129	0.694	54.0	0.341	—
Bemiss (1858): abnormality	.1067	5.792	54.3	0.498	0.53

larger recessive component for abnormalities. The number of detrimental equivalents expressed as conspicuous abnormality is about 1 or 2 per gamete, and probably larger under rigorous conditions. Most of these abnormalities are not congenital, and from Schull's sample it would appear that recessive genes are relatively less influential among congenital malformations than among defects with later onset.

Morton, Crow, and Muller (1956) concluded from studies of lethal genes in Drosophila that about 2 per cent of the genetic load per zygote will be expressed each generation. By the same argument, and taking the genetic load as 1.5 detrimental equivalents per gamete, we calculate that the expressed genetic load is about 2(.02)(1.5), or .06 detrimental equivalents per zygote. Comparison with the values of A in table 2 suggests that a substantial fraction of abnormality in nonconsanguineous marriages is attributable to heterozygous or homozygous effects of the same genes that cause abnormality as homozygotes in consanguineous marriages.

Retrospective studies.—The above samples are exceptional in that consanguinity was ascertained first and then morbidity was examined. Usually in human material the affected persons are first ascertained and their inbreeding then determined retrospectively. This method is the only feasible one if the trait is rare (Chung, Robison, and Morton, 1959).

For rare traits and for the small values of F which can be observed in man, the trait frequency at a given level of F may be written as

$$P = 1 - e^{-(A+BF)} \sim A + BF.$$

Let c_i be the frequency of the i^{th} value of F in the general population, $\alpha = \sum c_i F$ be the mean value, and $\sigma^2 = \sum c_i F_i^2 - \alpha^2$ be the variance of F in the population. Then the trait incidence is

$$I = 1 - \sum c_i e^{-(A+BF_i)} \sim A + B\alpha.$$

The mean inbreeding coefficient of ascertained abnormals (probands) is

$$\bar{F} = \sum c_i F_i (A + BF_i) / \sum c_i (A + BF_i) = A\alpha + B(\sigma^2 + \alpha^2)/I.$$

Solving these two simultaneous equations for A and B, we find

$$A = I - B\alpha$$

$$B = I(\bar{F} - \alpha)/\sigma^2$$

If I, α, and σ^2 are estimated reliably, and the standard error of $\bar{F}$ is σ_F, then the standard errors of A, B, and A + B are

$$\sigma_A = \alpha\sigma_B$$
$$\sigma_B = I\sigma_{\bar{F}}/\sigma^2$$
$$\sigma_{(A+B)} = (1 - \alpha)\sigma_B$$

These solutions may be regarded as first approximations (and in our experience very good ones) to the fully efficient maximum likelihood estimates, which are obtained iteratively from the system of equations

$$U = (A/I) \sum m_i(F_i - \alpha)/(A + BF_i)$$
$$K = (A/I)^2 \sum m_i[(F_i - \alpha)/(A + BF_i)]^2$$
$$B^* = B + U/K$$
$$A^* = I - B^*\alpha$$

where U is the maximum likelihood score for B, K is its variance, and A*, B* denote improved estimates from A, B. At the maximum likelihood value the standard errors are

$$\sigma_A = \alpha\sigma_B$$
$$\sigma_B = \sqrt{1/K}$$
$$\sigma_{(A+B)} = (1 - \alpha)\sigma_B$$

This extension of the theory of lethal and detrimental equivalents to retrospective ascertainment of consanguinity allows us to analyse all traits with a recessive component.

The results are especially interesting if we can isolate the contribution of completely penetrant, recessive genes. For then

$$A = \sum q^2$$
$$A + B = \sum q$$

Letting Q be the mean gene frequency per relevant locus and Δ^2 be the variance of q, we may write

$$A = \sum q^2 = n(Q^2 + \Delta^2)$$
$$A + B = \sum q = nQ,$$

where n is the total number of loci or complementary alleles producing the trait. Then n is at least $(nQ)^2/n(Q^2 + \Delta^2) = (A + B)^2/A$. The standard error of this estimate of n is about $\sigma_n = 2\sigma_B/Q$.

The mean gene frequency Q is $\sum q/n$, which is not more than $A/(A + B)$. The standard error of this estimate of Q is about $\sigma_Q = \sigma_B/n$.

In order to isolate the component due to completely penetrant, recessive genes, it is usually necessary to separate sporadic cases (mutants, phenocopies, poly-

genic cases, etc.) from the recessive ones. This presents a difficulty, for although it may be possible to show that familial cases from normal parents are recessive, the isolated cases may be a mixture of sporadic types (of different origin from the familial cases) and chance isolated cases (of the same origin as familial cases). Methods to investigate this model exhaustively have been developed as maximum likelihood scores (Morton, 1959). These involve far too many computations for desk calculation, but may be applied by means of the SEGRAN program for the IBM 650. (This program, written by Nancy S. Jones, may be obtained through the Department of Medical Genetics, University of Wisconsin.) So far this corpus of theory has been applied to three conditions, limb-girdle muscular dystrophy, deaf mutism, and low-grade mental defect.

Morton and Chung (1959) were able by a combination of genetic analysis and discriminant functions to divide a large sample of muscular dystrophy cases into three clinical and genetic types: dominant facioscapulohumeral, sporadic and recessive limb-girdle, and sex-linked Duchenne. Of limb-girdle cases, 41 per cent are sporadic, the remainder being due to highly penetrant, autosomal recessive genes. This mixture of sporadic and recessive limb-girdle types was revealed not only by SEGRAN, but also by the much lower inbreeding coefficient for isolated probands, many of which were sporadic, than for familial probands, which were shown to be recessive ($F = .00119$ and $.00804$, respectively).

Stevenson and Cheeseman (1956) studied the congenital deaf mute population of Northern Ireland. Their data were reanalysed by Krooth (1957) and Slatis (1958), with conflicting results. Chung, Robison, and Morton (1959) used SEGRAN to resolve the material into four components, due to recessive genes, dominant genes from affected parents, dominant mutants, and sporadic cases from infection or more complex genetic mechanisms, in the ratio 68:7:15:9. Among isolated cases the inbreeding coefficient was .00278, while among familial cases from normal parents, which were shown to be recessive, the inbreeding coefficient was .00854, confirming the genetic evidence for sporadic cases.

Penrose (1938) reported a family study of 1280 institutionalized mental defectives in Colchester, England. This classic investigation has never been excelled, but modern methods of analysis extract more information from such material than was possible at that time. Preparatory to a genetic study of low-grade mental defectives in Wisconsin, William Dewey in my laboratory reanalysed the old Penrose data (Dewey and Morton, 1960). We wanted to avoid polygenic, dominant, and cultural defectives and the possibility that dull or defective persons would mate preferentially with relatives. Therefore we considered only low-grade defectives (i.e., idiots and imbeciles, I.Q. less than 50), both of whose parents were of normal or superior intelligence (I.Q. greater than 85), excluding mongols, hydrocephalics, and cases due to known trauma, neoplasm, or infection. SEGRAN demonstrated that 87 per cent of admissible low-grade defectives were sporadic, and the remainder were due to highly penetrant, recessive genes. This conclusion was corroborated by the gross difference between the inbreeding coefficients of isolated cases ($F = .00097$) and familial cases ($F = .01349$).

It is of considerable interest that, for all three of the above conditions, the model of a mixture of sporadic and simple genetic types is valid. Presumably it applies to a very wide class of traits in man, such as congenital heart disease, cleft palate, cretinism, and a host of morphological and biochemical disorders, many of which have seemed to defy precise genetic analysis. This model has profound consequences for genetic risks, since if p is the risk in high-risk families, the probability that an isolated case with S normal sibs be sporadic is

$$x/[x + (1 - x)(1 - p)^S],$$

which is strongly dependent on sibship size, a factor which has almost never been taken into account in the specification of genetic risks.

The segregation load.—As indicated in table 4, about one gamete in four carries a gene which, if homozygous, would produce limb-girdle muscular dystrophy, deaf mutism, or low-grade mental defect. According to one hypothesis, this is a segregation load maintained by heterozygote advantage. In its extreme form, the theory postulates that, at each relevant locus, all homozygotes produce the trait in question, while heterozygotes are normal. This obviously requires a large number of mutually heterotic alleles, at least B/A alleles per locus by Crow's theorem (1958), or more than 200 for each of these conditions This is much larger than n, the total number of contributory loci or complementary alleles. Since local human populations in the past were not of greater order than this, it is incredible that selection against rare homozygotes could maintain such a high number of alleles at each locus. Moreover, the average allele frequency would have to be less than B/(B/A), or A, which is of the same magnitude as the mutation pressure, and so small that nearly every affected person would be expected to come from close consanguineous marriages. For all these reasons, the hypothesis of a segregation load in which all homozygotes are similarly affected is clearly inadmissible.

If some homozygotes are selected against in ways other than by the trait in question the argument based on the ratio B/A does not apply, but we can resort to a more general theorem. Wright (1949) showed that for any number of mutually heterotic alleles with the same fitness in heterozygotes, equilibrium is reached when $q_i = 1/s_i \sum (1/s_i)$. The expressed load in a randomly mating population is $\sum s_i q_i^2 = 1/\sum (1/s_i) = s_i q_i$. Accordingly, if at any locus there is one allele (or set of noncomplementary alleles) producing a gross defect in double dose, and any number of alleles which are manifested differently, we can determine the expressed segregation load at that locus from the frequency and selective disadvantage of the extreme allele alone. Since the load for any number of nonsynergistic loci is simply the sum of the separate loads, we can calculate from $\sum q$ in table 4 and the s values in table 5 that, if the load is segregational, the expressed load for limb-girdle muscular dystrophy, deaf mutism, and low-grade mental defect is approximately .22. This is an incredible intensity of selection when we consider that only about 100 loci are represented in these data, while the total number of loci per gamete is probably at least 10,000. Even if we ignore the load due to mutation and exogenous factors, the expressed load would

be equivalent to selective elimination through death or infertility of nearly all zygotes formed. This appears to refute the suggestion of Penrose (1949) that recessive genes for low-grade mental defect and other abnormalities are maintained as a segregation load through heterozygote advantage.

Prof. J. F. Crow has suggested a generalization of the above theorem, which removes the assumption that all heterozygotes are equally fit. Let $1 - s_{ij}$ be the fitness of genotype G^iG^j relative to the best genotype and let q_i be the frequency of $G^i(i = 1, 2, \cdots, k)$. The general formula for selection pressure (Wright, 1949) is $\Delta q_i = q_i(W_i - \overline{W})/\overline{W}$, where $\overline{W}$ is the mean fitness of the population and W_i is the mean fitness of G^i. In a randomly mating population,

$$\overline{W} = \sum_{i,j} q_i q_j (1 - s_{ij}) \qquad \text{and} \qquad W_i = \sum_j q_j (1 - s_{ij}).$$

At equilibrium, $W_i = \overline{W}$, or $\sum_j q_j s_{ij} = \sum_{i,j} q_i q_j s_{ij}$, which by definition is the expressed genetic load. Since $q_i s_{ii}$ is less than or equal to $\sum_j q_i s_{ij}$ for all k alleles, it follows that

$$\sum q_i s_{ii} \leq k \sum_j q_i s_{ij}$$

or $q_i s_{ii} \leq 1/k$ total genetic load $\leq$ expressed genetic load.

Thus for a locus with k alleles maintained by selection in a randomly mating population, with no restriction on the selection coefficients, the product of any gene frequency and its selection coefficient in the homozygote is always less than or equal to the expressed genetic load at that locus, and the total genetic load is less than or equal to k times the expressed load.

It can be verified that this result holds a fortiori for the model of squared deviations from an optimum and genes that are additive (Wright, 1935). For if the mean and optimum coincide, the chance of a defect is proportional to the genetic variance on the primary scale, or $B(1 + F)$, and the ratio B/A cannot be greater than 1.

These arguments appear to rule out explanations of inbreeding effects on morbidity in terms of the segregation load or any other purely selective system. If, on the other hand, inbreeding effects are due to the mutational load, the expressed effect will be only about $Q + F + 2h$ as great as if the load were segregational, and B/A will be increased inversely (Crow, 1958). These expectations are consistent with the available data.

This is the only instance in any organism, except laboratory populations of Drosophila, where it has been shown that the genetic load is largely not segregational. The evidence from Drosophila depends on the assumption that heterozygote effects measured in the laboratory are representative of effects in nature. The human data are not subject to this reservation, since they do not depend on measurement of heterozygote effects. The method is applicable to other rare traits, where it can be shown that the recessive genes in question have high penetrance. It is hardly to be expected on physiological grounds that hypomorphic mutants would often be maintained by heterozygote advantage, and most recognized mutants are hypomorphic, with products that function with reduced

efficiency compared with the products of adaptive alleles. Therefore balanced polymorphism is probably limited to a small fraction of loci, each of which may account for an appreciable fraction of the expressed genetic load, but a negligible fraction of the total genetic load measured by inbreeding.

The discussion so far has dealt only with the cases that can be shown to be recessive, and would seem at first to leave open the possibility that sporadic cases represent balanced polymorphic systems and are therefore part of the segregation load. Lerner (1954) has in fact postulated from studies on long-continued inbreeding in chickens that this load is brought to expression as sporadic "phenodeviants", which are the price an organism pays for falling short of the "obligate level of heterozygosity". The experimental evidence is exceedingly complex, involving inbreeding and unavoidable selection over many generations, with the possibility of mutation, recombination, and other phenomena that are impossible to predict and difficult to analyse. Any rigorous examination of this theory must be made in simpler material, preferably by producing different levels of inbreeding in a single generation without selection.

Lacking definitive experimental evidence, we can still try to formulate the theory in a way that will permit a critical test. One possible interpretation is that sporadic cases are due to homozygosity at many loci simultaneously. Suppose that n loci are involved, and the frequency of the recessive allele is q at each of them. Then the frequency of the multiple homozygote will be

$$[(1 - F)q^2 + Fq]^n$$

which equals $q^n[q + pF]^n$, or

$$q^n[q^n + npFq^{n-1} + n(n - 1)p^2F^2q^{n-2}/2 + \cdots + p^nF^n],$$

which is q^{2n} if $F = 0$ and q^n if $F = 1$, so that with this model the trait frequency under inbreeding cannot be greater than the square root of its frequency under random mating. If q is small, the inbreeding effect will be large and markedly nonlinear, while if q is large, the multiple homozygote and the trait it determines will be common. If $q = \frac{1}{2}$, the frequency of the trait will be

$$(\tfrac{1}{4})^n[1 + nF + n(n - 1)F^2/2 + \cdots + F^n],$$

which gives an appreciable inbreeding effect if n or F is large, in which case the nonlinear component will also be large. If q is large and n is small, the trait will be common and familial and B/A will be small. If n is large, the trait will be vanishingly rare. These expectations are not borne out by the data.

Another possible interpretation of the phrase "obligate level of heterozygosity" is that phenodeviants require homozygosity at single loci as a necessary condition, but will be produced only if, in addition, the residual genotype is inbred. Suppose that at the primary locus the frequency of the recessive allele is q, and the probability that a homozygote will express the trait is cF. Then the frequency of the trait will be $cqF(q + pF)$, which again is markedly nonlinear on F if F is large relative to q. Both ways of interpretating Lerner's theory imply a more than linear increase of phenodeviants with inbreeding.

Recently Neel (1959) has suggested that many sporadic malformations in man may be phenodeviants, or "segregants resulting from the existence and functioning of complex (multilocal) genetic homeostatic systems, of the type particularly discussed by Lerner (1954)". However, it is not at all apparent that the heritable defects which characterize highly inbred lines (phenodeviants in the sense of Lerner) have any real similarity to sporadic defects in randomly mating populations (phenodeviants in the sense of Neel). By Lerner's criteria of increased incidence under inbreeding and under unfavorable environments, most of the malformations studied by Neel can hardly be considered phenodeviants, since they are at the same frequencies in the more inbred Japanese and less inbred Caucasian populations and are perhaps least frequent under the poor environment of American Negroes. Neel cites the following facts in support of his interpretation.

1) Total malformation rates are substantially the same in different populations.
2) Specific malformations have different frequencies among populations.
3) Type-specific malformation rates are slightly increased in sibs of probands.
4) Monozygotic concordance is low.
5) Malformation rates in the U. S. are intermediate between England, Switzerland, and Sweden.
6) Malformation rates increase with inbreeding.
7) Specific malformation rates depend on sex, parental age, and associated defects.

It is difficult to see how these observations bear on the phenodeviant hypothesis. Fact (6) indicates that some malformations depend on homozygosity, fact (4) indicates that most malformations depend on special environmental circumstances, and the other points can be interpreted in a great variety of ways, and are therefore irrelevant.

To support Neel's hypothesis that a large proportion of sporadic malformations are phenodeviants, it will be necessary to show that

1) They are genetic.
2) They are polygenic.
3) They depend on homozygosity.
4) The genes involved are maintained through heterozygote advantage.

Neel offers no critical evidence for any of these points, and it would seem that his hypothesis must be stated more clearly before a definite test is possible. Does he assert that *all* the genes which produce phenodeviants are maintained by heterozygote advantage, or that only a "significant fraction" are? In the latter case, if the significant fraction is sufficiently small, the hypothesis is neither disputable nor heuristic. Would a gene maintained by mutation pressure, with modifiers maintained by heterozygote advantage, satisfy his concept of a phenodeviant or not? And does he espouse Lerner's mystical thesis that phenodeviants cannot in principle be referred to any specifiable set of loci, but represent the effect of too high a level of homozygosity *per se* (an hypothesis that would require that *all* types of phenodeviants be increased in affected individuals and their sibs)? This leads to the following dilemma. Suppose phenodeviant A occurs

when more than n_A loci become homozygous, and phenodeviant B when more than N_B loci are homozygous. Then if n_A is greater than n_B, defect B should always accompany A, while if n_B is greater than n_A, defect A should always accompany B. Extending this to all defects, the one which requires the highest level of homozygosity, and therefore is most sensitive to inbreeding, must be accompanied by all the others which are less sensitive to inbreeding. Clearly this prediction is not true. Thus there is not sufficient information in the level of homozygosity *per se* to account for the specificity and distribution of defects.

In view of the extremely low penetrance of phenodeviants which must be postulated to fit the twin discordances, Neel's vague and complex hypothesis can neither be rigorously supported nor disproved at the present stage of human genetics. However, it does seem important (and critical evidence that the load represented by inbreeding is not a segregation load) that the inbreeding coefficient for isolated cases of limb-girdle muscular dystrophy, deaf mutism, and low-grade mental defect agrees quantitatively with the assumption that sporadic cases are not associated with inbreeding. In fact, the estimate of the proportion of sporadic cases based on this assumption and the inbreeding coefficients of familial and isolated cases is slightly, but not significantly, higher than the estimate from SEGRAN, whereas just the opposite would be expected if sporadic cases were associated with inbreeding (table 3). Since sporadic cases are not demonstrably related to homozygosis, the hypothesis of phenodeviants in man must be dismissed as either false or operationally undefined. Most sporadic cases are probably due to maternal and environmental factors and to a variety of nonrecessive genetic mechanisms, including polygenes, heterozygotes with low penetrance, dominant mutations, deletions, and aneuploidy.

The mutational load.—Having shown that the genetic load revealed by inbreeding is largely not a segregation load, we are permitted to assume that it is mutational. At equilibrium, the mutation rate per relevant locus is

$$u = s[q^2(1 - \alpha') + q\alpha' + hq(1 - q)(1 - \alpha')].$$

where α' is the inbreeding coefficient under which the population reached equilibrium. For small q, h, and α',

$$u = s(q^2 + q\alpha' + qh).$$

Summing over all loci, the mutation rate per gamete with s constant is

$$\begin{aligned} U &= s[\sum q^2 + \alpha' \sum q + \sum qh] \\ &= s[A + \alpha'(A + B) + \sum qh] \end{aligned}$$

TABLE 3. ESTIMATES OF THE PROPORTION OF SPORADIC CASES BASED ON INBREEDING AND SEGREGATION

Trait	Inbreeding estimate	Segregation estimate
Limb-girdle muscular dystrophy	.500	.413
Deaf mutism	.342	.221
Low-grade mental defect	.888	.868

TABLE 4. ESTIMATES OF I, Σq, AND $\Sigma q^2 \times 10^5$

	All cases, I	Recessive cases, I	Σq	Σq^2
Limb-girdle muscular dystrophy	7	4	800	3
Deaf mutism	31	21	8,000	18
Low-grade mental defect	362	48	16,700	41
Total	400	73	25,500	62

TABLE 5. ESTIMATES OF MUTATION RATES, GENE FREQUENCIES, AND NUMBERS OF LOCI

	Selection Coefficient s	Mean gene frequency Q	Number of loci n	Mutation rate per gamete $U \times 10^5$	Mutation rate per locus $u \times 10^5$
Limb-girdle muscular dystrophy	.75	.0044	2	6	3.4
Deaf mutism	.68	.0023	35	45	1.3
Low-grade mental defect	.97	.0024	69	137	2.0

The value of α' is almost certainly less than .02 (Wright, 1950), and is believed to be about .006 (Neel et al., 1949). Assuming h smaller than α', we may neglect the term in h. Table 5 gives this estimate of U and of $u = U/n$, which is remarkably similar to estimates of per locus rates by other methods. (In principle, u tends to be overestimated since n is the minimum number of loci, while the estimate of U is unbiased, with a standard error of about $s\alpha'\sigma_B$.) The standard error of the estimate of u is about $s(2Q + \alpha')\sigma_B/n$.

Morton, Crow, and Muller (1956), assuming that the frequencies of detrimental genes are largely determined by selection in heterozygotes, concluded that the mutation rate is approximately

$$U = s(.02) \sum q \text{ per gamete, or}$$

$$u = s(.02)Q \text{ per relevant locus,}$$

for a class of mutants with constant homozygote disadvantage s and 2 per cent dominance. Applied to the data of tables 4 and 5, this gives estimates about twice as large as those obtained on the assumption of complete recessivity. An intermediate value is obtained if heterozygote effects are limited to occasional penetrance as sporadic cases, even if all sporadic cases are attributed to this cause. Thus the mutation rate estimates are fairly stable for a range of assumptions about dominance.

DISCUSSION

Through this extension of the theory of lethal and detrimental equivalents, it becomes possible to treat all traits with a rare recessive component affecting either viability or fertility. On the simplest assumptions, fertility is related to inbreeding as e^{A-BF}, where B is the number of sterile equivalents revealed by inbreeding and e^A is the fertility in a randomly mating population. Experiments are in progress in my laboratory to test this and other aspects of the theory in Drosophila.

Evidence that full lethals are a major component of lethal equivalents in natural populations, (Slatis, 1960, Greenberg and Crow, 1960), suggests that changes in sex ratio and inbreeding effects in an irradiated population are largely due to lethal genes. This prediction has been confirmed in Drosophila (Friedman, Morton, in preparation), and is being tested in the rat (Chapman, in preparation). These experimental results provide justification for several applications to man.

Firstly, it should be possible to estimate the radiation dose which doubles the mutation rate. In Drosophila (Oster, 1959) and in the mouse (Russell et al., 1958) the doubling dose is greater for chronic than acute gonial radiation, and greater for gonial than gametic stages. The doubling dose for acute gonial radiation in the mouse has been found to be about 35 r (Russell et al., 1958). The data on sex ratio collected by the Atomic Bomb Casualty Commission in Hiroshima and Nagasaki provide an estimate of radiation effects as lethal equivalents per gamete (table 6). The relation between sex ratio and exposure to the atomic bombs was first considered significant (Neel et al., 1953), then nonsignificant (Neel and Schull, 1956), then significant again (Schull and Neel, 1958). We assume that the ratio of males to females is $pe^{-mR}/(1 - p)$ following a dose of R roentgens to oogonia, where m is the mutation rate in lethal equivalents per r per X chromosome. Since the X chromosome is about 5.75 per cent of the haploid genome of man (Chu and Giles, 1959), the mutation rate per gamete per r may be taken as approximately m/.0575. Accepting the Japanese data at face value, we estimate $m = 2.3 \times 10^{-4}$. Therefore the doubling dose is calculated to be the spontaneous rate, about 0.10 lethal equivalents, divided by the estimate of m/.0575, or 25 r, with a large standard error.

In principle, the doubling dose can be estimated from data on populations at equilibrium under two or more levels of chronic radiation. The expressed load is $A + BF + CR + DFR$, and the dose which doubles the total load is $(A + B)/(C + D)$, while the dose which doubles the recessive load is B/D. This is the dose required to double the mutation rate providing migration is negligible, otherwise it is an upper estimate of the doubling dose.

If N zygotes are drawn from a population that received a fraction x of the doubling dose, the number of induced mutants is 2xNU, where U is the spontaneous mutation rate per gamete. Repeating this dose each generation, an equilibrium will be established when the incidence of affected persons is increased by xU for recessive defects and 2xU for dominants. From table 5, we can calculate that administration of a doubling dose for one generation to a population from which 10^6 zygotes are drawn would produce about 120 mutations to genes for limb-girdle muscular dystrophy, 900 mutations to genes for deaf mutism,

TABLE 6. SEX RATIO AMONG CHILDREN OF SURVIVORS OF ATOMIC BOMBS
(Data of Schull and Neel)

	Maternal dose (r)				
	0	8	75	100	200
Number	53691	29978	5853	355	3041
Male/female	1.089	1.079	1.053	1.076	1.046

and 2740 mutations to genes for low-grade mental defect, as well as countless mutations of other kinds, amounting in all to about 200,000 lethal equivalents, only a small fraction of which would be expressed the first generation. If this radiation were repeated each generation until a new equilibrium was reached, we would expect an increase of at least 60 cases of limb-girdle muscular dystrophy, 450 cases of deaf-mutism, and 1370 cases of low-grade mental defect per million of the population, in addition to other defects, amounting in all to about 200,000 lethal equivalents, (Morton, Crow, and Muller, 1956). These estimates of radiation effects on muscular dystrophy, deaf mutism, and low-grade mental defect are minimal, since they ignore dominant and sporadic cases, which will also increase with radiation.

At the present time the total genetic load is operationally defined only for recessive genes, and we have little information on radiation response for hyperploidy, polygenic variation, or epigenetic factors in any organism, certainly not in man. Therefore estimates of the total genetic effects of radiation are subject to serious and uncontrollable errors. A case in point is the attempt of Carter (1957) to estimate the effect of radiation on the incidence of mongolism, assuming the same doubling dose as for recessive genes. We know now that most cases of mongolism are due to trisomy, (Lejeune et al., 1959), the radiation response for which is presently conjectural. Penrose (1959) has stigmatized such calculations as follows: "It would be a serious matter if superficial compilations of this sort were taken to indicate the modern standards of scientific work in human genetics. The question which the analysis seeks to answer is basically unsatisfactory. We cannot estimate how much disease is genetical in origin." Such ambitious estimates of total genetic effects should not be confused with the approach to recessive genes through lethal and detrimental equivalents. Except for a few estimates of dominant and sex-linked mutation rates in man and extrapolations from other organisms, the theory of lethal and detrimental equivalents presents the only good evidence on human mutation rates, and the most powerful and rigorous method for extending this evidence.

SUMMARY

It has been estimated from studies of increased mortality in children of consanguineous marriages that the average person carries heterozygously the equivalent of 3–5 recessive lethals acting between late fetal and early adult stages, due to a mutation rate to such genes of .03–.05 per gamete per generation. This theory of lethal equivalents has now been extended to detrimental traits, not measured as mortality, for which consanguinity may be ascertained either prospectively, before studying morbidity, or retrospectively as a family study of ascertained cases (probands). Data on malformations reveal about 1.5 detrimental equivalents per gamete, which cause about 6 per cent defects per zygote in a randomly mating population. When it is possible by appropriate methods to isolate the components due to highly penetrant recessive genes, the theory simplifies greatly, and leads to a distinction between mutational damage (the mutation load) and segregation from superior heterozygotes (the segregation load).

Evidence on deaf mutism, limb-girdle muscular dystrophy, and low-grade mental defect indicates that most of the genetic load revealed by inbreeding is due to mutational damage, occurring at predictable rates at a determinable number of loci. About one gamate in four carries a gene which, if homozygous, would produce one of these defects. Sporadic cases are not associated with inbreeding. Implications of these results for studies of induced mutation are discussed.

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"Genetics and Twentieth Century Darwinism" A review and discussion[1]

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THIS symposium on the centenary of the publication of Darwin's "Origin of Species" gives a broad sample of current observational and experimental research that bears on evolution. The introductory address by Mayr "Where are we?" was intended to set the stage. He discussed the history of population genetics since 1900, the contributions of various lines of research and unsolved problems. As it seems to me that he has seriously misinterpreted the roles of these various lines, as well as the contributions of the one with which I am most familiar, I will single this out for special discussion later.

There followed twenty-four papers, largely on special researches. In the concluding address by Stebbins, "The synthetic approach to organic evolution", he listed seven major points on which he believed there is general agreement. He also listed a number of unsolved problems and brilliantly summarized the results of the symposium by bringing out how the various papers bore on these problems.

It is not practicable to summarize, and much less to discuss the specific contributions here. The reader may be given some idea of the scope by a list of papers not already referred to.

Dobzhansky, Th. Evolution of genes and genes in evolution.

Stubbe, Hans. Considerations on the genetical and evolutionary aspect of some mutants of *Antirrhinum, Hordeum* and *Lycopersicon.*

Buzzati-Traverso, A. A. Selection, quantitative traits and polygenic systems in evolution.

Morley, F. H. W. Natural selection in relation to ecotypic and racial differentiation in plants.

Mourant, A. E. Human blood groups and natural selection.

Lamotte, M. Polymorphism in natural populations of *Cepaea nemoralis.*

Carson, H. L. Genetic conditions which promote or retard race formation.

Schwanitz, Franz. Selection and race formation in cultivated plants.

Barnicot, N. A. Darwin's view on the evolution of human races in the light of modern research.

Sheppard, P. M. The evolution of mimicry; a problem in ecology and genetics.

Ehrendorfer, F. Differentiation-hybridization cycle and polyploidy in *Achillea.*

Coon, C. S. Race and ecology in man.

[1] Cold Spring Harbor Symposia on Quantitative Biology Vol. 24.

[2] Paper no. 778 from the Department of Genetics, University of Wisconsin.

Kitzmiller, J. B. and Laven, H. Speciation in mosquitoes. 1. Race formation and speciation in mosquitoes (Kitzmiller; 2. Speciation by cytoplasmic isolation (Laven); 3. Evolutionary mechanisms (both authors).
Baker, H. G. Reproductive methods as factors in speciation in flowering plants.
Wallace, Bruce. The influence of genetic systems on geographical distribution.
Kurten, B. Rates of evolution in fossil mammals.
Andrews, H. N., Jr. Evolutionary trends in early vascular plants.
Heberer, G. The descent of man and the present fossil record.
Hunt, Edward E., Jr. The continuing evolution of modern man.
Simpson, G. G. The nature and origin of supraspecific taxa.
Smith-White, S. Chromosome evolution in the Australian flora.
Rensch, B. Trends towards progress of brains and sense organs.

Mayr begins with a division of the history of the subject since 1900 into three parts:

(1) *The Mendelian Period.* "The period from 1900 to about 1920 saw a sharp cleavage, an almost bridgeless gap, between the evolution-minded naturalists on the one hand and the experimental geneticists on the other hand" (selectionist and mutationist, respectively).

(2) *Classical Population Genetics.* After referring to publications of Fisher (1930), Wright (1931) and Haldane (1932) to indicate what he is talking about, Mayr writes as follows:

"The emphasis in early population genetics was on the frequency of genes and on the control of this frequency by mutation, selection and random events. Each gene was essentially treated as an independent unit, favored or discriminated against by various causal factors. In order to permit mathematical treatment numerous simplifying assumptions had to be made, such as that of an absolute selective value of a given gene. The great contribution of this period was that it restored the prestige of natural selection, which had been rather low among the geneticists active in the early decades of the century, and that it prepared the ground for the treatment of quantitative characters. Yet this period was one of gross oversimplification. Evolutionary change was essentially presented as an input or output of genes, as the adding of certain beans to a beanbag and the withdrawing of others. This period of "beanbag genetics" was a necessary step in the development of our thinking, yet its shortcomings became obvious as a result of the work of the experimental population geneticists, the animal and plant breeders, and the population systematists, which ushered in a third era of evolutionary genetics."

(3) *The Newer Population Genetics.* Mayr describes this as follows, immediately after the above quotation:

"The next advance was characterized by an increasing emphasis on the interaction of genes. Not only individuals but even populations were no longer described atomistically as aggregates of independent genes in various frequencies, but as integrated, coadapted complexes. A gene is no longer considered to have one absolute selective value, but rather a wide range of potential values that may extend from lethality to high selective superiority, depending on

genetic background and on the constellation of environmental factors. I have referred to this new mode of thinking as the genetic 'theory of relativity'. Dobzhansky's 'balance theory' of genetic variation is one of its aspects. The thinking of this newer population genetics is in considerable contrast to that of the classical population genetics and even more so to that of early Mendelism. This change of view is not always realized, even by professional geneticists who have no contact with population genetics."

"THE MENDELIAN PERIOD"

Returning to Mayr's first period my recollections of the attitudes of naturalists and geneticists toward natural selection during the latter half of it differ considerably from Mayr's statement. A very thorough and objective account of the various theories of evolution that had developed before any serious contamination by Mendelism, is given in Kellogg's "Darwinism Today" published in 1907, which I read in 1910. The impression is that of almost universal dissatisfaction with natural selection as the major principle but utter chaos with respect to substitutes. There was a bewildering array of names of theories but most of them referred to variants of Lamarckism, orthogenesis or heterogenesis. This is not surprising as there was no possibilty of settling the matter in the absence of sound knowledge of heredity. Yet, even as late as 1934, H. F. Osborn, the leading American paleontologist, probably spoke for most naturalists in holding that natural selection was inadequate.

Heterogenesis had, of course, much influence in early genetic thinking in the form of de Vries' mutation theory. Even this, however, was not as antagonistic to natural selection as the other theories. de Vries wrote in 1906 "Not withstanding all these apparently unsurmountable difficulties, Darwin discovered the great principle which rules the evolution of organisms. It is the principle of natural selection . . . It is the sieve which keeps evolution on the main line, killing all or nearly all that try to go in the other direction. By this means, natural selection is the one directing cause of the broad lines of evolution." By 1910 at least, most geneticists were much more Darwinian than de Vries and those with whom I had most contact (Castle, East) were wholly Darwinian. The development of the multiple factor theory of quantitative variability by Nilsson-Ehle, East, and Shull supplied admirably the basis for this view.

It is true, however, that most geneticists were more concerned with the kinds of mutations that are significant in evolution than with the process. Evolution is something that happens to populations and without a mathematical theory, connecting the phenomena in populations with those in individuals, there could be no very clear thinking on the subject. As to the kinds of mutations it has of course turned out that chromosome aberrations (from which de Vries was generalizing without knowing it) and both conspicuous and inconspicuous gene mutations are utilized in evolution though I think that it is generally agreed now that the last are most important in character change (Stebbins' third point).

Population genetics in a broad sense began well before 1900 in the efforts of Galton, Pearson and the rest of the Biometric school to devise tools for describing adequately the characteristics of populations, consisting of individuals each with a unique array of characters. Unfortunately for them, they went further and tried to deduce the nature of heredity from such population characters as the correlation between relatives. Shortly after 1900, experimental genetics and population genetics confronted each other in the persons of Bateson and Pearson across a seemingly "bridgeless gap."

A bridge was, however, inadvertently constructed by Pearson in 1904 in a paper in which he ruled Mendelism out because of a discrepancy between the genetic correlations expected under it and those actually observed. Yule (1906) showed that this discrepancy was based merely on a too rigid acceptance of complete dominance as a principle. Hardy, and Weinberg in 1908 extended Pearson's bridge, and Weinberg by 1910 had brought about a rather complete synthesis of the two kinds of data for homogeneous static populations. Fisher (1918) did the same from a somewhat different mathematical viewpoint. It is interesting to note that his most important extension was a more adequate treatment of factor interaction.

Another aspect of population genetics, the experimental study of the effects of inbreeding, crossbreeding and selection on laboratory populations goes back to Darwin's studies of inbreeding and crossbreeding in plants. There were several important studies of inbreeding in mammals in the latter part of the 19th century. Such experiments became more significant after 1900, when they could be given a Mendelian interpretation. Castle's experiments on inbreeding in Drosophila and on the effects of selection in hooded rats were among the most noteworthy. My own entry into population genetics, apart from serving as Prof. Castle's assistant (1912–15) in his selection experiment, came on taking charge (1915–25) of an extensive project on the effects of inbreeding and crossbreeding in guinea pigs, started in 1906 in the U. S. Bureau of Animal Industry (Wright 1922b).

Still another aspect of population genetics is exemplified by Sumner's (1918) very instructive study of the genetics of differences within and between subspecies of the wild species, *Peromyscus maniculatus*.

This brings us to Mayr's second period to which he confines mathematical population genetics in a Procrustean bed by lopping off all work before 1920 and all of the great expansion by many investigators after 1940.

"CLASSICAL POPULATION GENETICS"

The results which I obtained from the study of inbreeding and crossbreeding in guinea pigs agreed well with those of most others with other organisms and could be given a Mendelian interpretation. I was led into the mathematical aspect by the effort to develop a more general theory than that arrived at by Jennings (1916) for special cases, a theory applicable to any sort of population structure (Wright, 1921). The inbreeding coefficient (1922a,b) that was implied made it possible to generalize the Hardy-Weinberg rule to situations

other than random mating. The dynamic aspects of inbreeding and assortative mating were also dealt with. The primary objective was to develop a theory on how best to combine inbreeding and crossbreeding with selection in livestock improvement. The only sort of selection considered was that directed toward an optimum, one that involves interaction in an extreme form. The mathematical analysis of this did not go far enough to be of much value until I came back to it in 1935.

Haldane (1924) made a more fruitful attack on the mathematical theory of selection by considering the case of a single gene subject to selection and mutation. This was a very important contribution to the dynamics of populations. If, however, nothing further had been developed in mathematical population genetics, Mayr's criticism would be sound. As it was, Haldane himself discussed selection under many complicating conditions including factor interaction (summarized in his 1932 book). The treatment of factor interaction was the central theme of the other two publications cited by Mayr. The situations treated in these were largely complementary.

The central theme of Fisher's book was the determination of the rate of increase of fitness (or mean selective value in my terminology) in a large homogeneous population, assuming that the effects of the genes may be involved in any sort of interaction whatever. His "fundamental theorem of natural selection" was that "the rate of increase of fitness of any organism at any time is equal to its genetic variance in fitness at that time" (in which genetic variance is defined as the additive component of the portion due to heredity). To demonstrate that progress by selection is restricted to the net effects of the genes in the combinations in which they enter is not to ignore interaction as Mayr seems to suppose. Actually Fisher's theorem holds only if the selective value of the total genotype is constant in any given environment (Wright, 1956) and under certain conditions selection may lead to decrease of mean selective value. Nevertheless the theorem covers a great deal of ground.

The objective of my papers in the 1930's was exploration of the ways in which selection may take advantage of favorable interaction effects most effectively. The most important case is that in which there are many separate peaks in the "surface" of selective values ($\bar{W}$), a case in which selection of the sort considered by Fisher merely holds the population to a single peak irrespective of whether high or low. This case includes that of selection directed toward an intermediate optimum, an almost universal situation for quantitative variability in nature, and also most cases of multiple interacting loci with pleiotropic effects. It may be noted parenthetically that Mayr states flatly that the symbol W for selective value in this theory represented an absolute value assigned to a given gene. Actually it has always been defined as applying to a *total* genotype in the system under consideration, thus involving whatever interaction effects there may be among the component genes (Wright, 1937).

The major question considered was whether there is "a mechanism by which the species may continually find its way from lower to higher peaks". One such mechanism is the production of clones since interclonal selection is ob-

viously according to the genotype as a whole. In cases in which clones are not produced to a significant extent (as in vertebrates, most insects and many higher plants) it was concluded that the only effective mechanism was subdivision of the species into small local populations (demes), sufficiently isolated to permit more or less random differentiation with respect to alleles with minor differential effects, but not too isolated for interdemic selection (by differential growth and migration).

This process requires that there be a balance among the pressures on each of the gene frequencies so as to permit two, or better multiple, alleles at fairly high frequencies at many, or better all, of the loci that are involved. Mayr seems to imply in his beanbag analogy that it was assumed that there is typically an approach to homozygosis at all loci in the species, except for the occasional occurrence of favorable mutations that ultimately displace the previous type genes. Such an assumption was of course wholly incompatible with my theory.

In the second place, the process requires that there be a balance between the systematic pressures at each locus, tending to maintain control by the same selective peak in a given deme, and random processes that permit the gene frequencies to keep changing somewhat, and occasionally so much that the system drifts across a shallow "valley" in the surface of selective values, to come under the control of another and higher peak. Continuing evolution requires that random drift be not so great as to bring about fixation of genes. Any fixation that occurs should be that brought about by the relatively strong selection that carries the system, after crossing a valley, to the neighborhood of the new controlling peak. The course of evolution of the species as a whole is then determined by interdemic selection.

In this theory, the joint effects of random drift and intrademic selection merely supply raw material for interdemic selection. Mayr treats the role of random drift in this theory as if it were the same as the random fixation of the earlier theories of Gulick and Hagedoorn. The error here is analogous to that of treating the role of mutation in supplying raw material for Darwinian selection as if it were the same as in the mutation theories of de Vries and Goldschmidt.

"THE NEWER POPULATION GENETICS"

Mayr attributes to the newer population genetics "increasing emphasis on the interaction of genes", the discovery of "integrated coadaptive complexes", recognition of "variable selective values of genes", "Dobzhansky's balance theory of genetic variation" and in a later paragraph than that quoted earlier "the interpretation of the inheritance of quantitative characters by Mather and the development of the more sophisticated modern views on the interaction of genetic factors, on coadaptation and on genetic homeostasis".

As just noted the treatment of interaction systems was the central theme of most of "classical" population genetics. The "harmonious" system of genes that characterizes a selective peak is certainly an "integrated coadaptive complex". Such a system has by the definition of a peak, Lerner's property of genetic

homeostasis and any coadaptive complex with genetic homeostasis is ipso facto located at a selective peak. Variable selective values were necessarily recognized in dealing with gene interaction. Mather's "polygenic" inheritance is basically the same thing as the multifactorial inheritance of Nilsson-Ehle, East and Shull. Mather's important contribution to the theoretical consequences of linkage is a part of mathematical population genetics. Dobzhansky's balance theory has at least elements in common with Fisher's theory of polymorphism and with one of the essential aspects of the balance theory of evolution that I developed.

What then has been the contribution of "the newer population genetics"? I think that Mayr has obscured this by his repeated treatment of mathematical population genetics and the genetics of natural populations as if they were alternative ways of making contributions of the same sort to evolutionary theory. Actually each plays a role in the synthetic theory that the other cannot possibly play. A synthesis is more than a list of contributions.

The role of the mathematical theory is that of an intermediary between the bodies of factual knowledge discovered at two levels, that of the individual and that of the population. It must deduce from the postulates at the level of the individual and from models of population structure what is to be expected in populations, and then modify its postulates and models on the basis of any discrepancies with observation and so on.

In developing the balance theory of evolution, I was trying to arrive at a judgment of the most favorable conditions for evolution under the Mendelian mechanism. No amount of mathematical analysis could prove that these conditions actually occur. The great achievement of the recent studies of natural populations has been to discover what natural populations are actually like, genetically. Dobzhansky's balance theory summarizes what he and his associates have actually found in nature and differs from abstract balance theories in concrete details that could hardly have been anticipated. I fully concur in Mayr's tribute to these tremendous achievements. Nobody, however, has understood better than Dobzhansky the reciprocal relation between concrete facts and abstract theory, as exemplified in his continual collaboration with mathematical population geneticists.

It is I think seriously misleading to divide the history of population genetics since 1900 into consecutive 20 year periods devoted respectively to the genetic mechanism, the mathematical deductions and the genetics of real populations. All of these strands have actually been developing simultaneously and in continual interaction since 1900. It is the binding together of the facts at the two levels of observation into a self-consistent theory, necessarily mathematical to a considerable extent, that constitutes the modern microevolutionary synthesis.

None of the three strands in this synthesis is complete. As we learn more of the general significance of such phenomena as the cytoplasmic heredity of Michaelis, Ephrussi and others, the activators and modulators of McClintock and Brink, paramutation of Brink, etc., these will have to be incorporated into the theory. The study of the effects of various patterns of selection in laboratory populations, in domestic animals, and cultivated plants still has far to go,

and this is much more the case with the genetics of natural species. We may anticipate surprises that will require readjustments all along the line. The present symposium supplies many new data, largely at the level of actual populations, which must now be digested.

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BOOK REVIEWS

Science and Liberal Education

By BENTLEY GLASS. Louisiana State University Press, 1959, x + 115 pp., $3.00.

THIS IS A BOOK to give to an unscientific colleague, which is what the editor of this journal did.

The first of the three lectures which constitute the volume, "Genetics in the Service of Man," shows the reader the power of science and startles him by drawing illustrations not from flashy and fashionable applied physics but from biology. It frightens the layman, too, for he can only agree that "power, especially unlimited power, can be more danger than boon."

Dr. Glass demonstrates first, however, what a boon it can be, showing the services of genetics in the development of greater food supply (hybrid corn) and in the development of "miracle drugs" (mass production of penicillin). And then he frightens us again with alternative pictures of the future that remind us of Swift's voyage to Luggnagg, the land of the Struldbrugs, in Book III of *Gulliver*, and of Aldous Huxley's *Brave New World*. We may have to contemplate, he tells us,

> "a future population composed largely of persons who must wear both glasses and hearing aids, and who must start the day by inserting their false teeth, taking first an insulin injection in one arm and then allergy shots in the other, and finally topping things off with a tranquilizing pill..."

This is Luggnagg. But if we undertake to control our own evolution, Mr. Glass reminds us, we may do worse:

> "Today man, in the shape of the geneticist as well as in the shape of the physicist, possesses more power than wisdom. He could, as I have said elsewhere, 'crystallize human society into a changeless rigidity dominated by reason armed with scientific knowledge.' He might even breed 'a ruling caste of a relatively few individuals, evolving higher and higher levels of intelligence, and a helot or robot class of workers, chained by their insticts and minimal intelligence to the performance of simple, mechanical tasks'."

This is the brave new world.

The rhetorical question, "Can the geneticist breed wisdom, or integrity, or even simple humanity?" with which Dr. Glass follows these sentences carries its own answer, but the theme of the book is also an implied answer. Wisdom, integrity, and simple humanity will be fostered by liberal education—not guaranteed by it, not achieved at all if we are bred wrong, but fostered by it. But whereas Dr. Glass succeeds admirably in persuading the humanist that science is powerful, valuable, and dangerous, deserving to be encouraged and feared, and requiring to be studied, he succeeds less well in persuading him that science should therefore be the "core" of "Liberal Education in a Scientific Age." It deserves this position, he tells us,

> "because science is concerned with the development of increasingly adequate concepts about man and man's place in the order of the universe"

and because

> "science has acquired the function of enlarging man's command over nature (including his own nature) by means of new knowledge acquired through systematic, accurate observation and controlled experimentation."

We can accept both these propositions, however, without following to the conclusion that science should become the "core" of the school curriculum, and we are even encouraged to reject this conclusion by the extent to which Dr. Glass uses the methods of rhetoric and poetry in his persuasion and turns philosopher in his argument.

First there is the disarming analogy:

"The core of the apple is certainly not the whole apple—not even the most beautiful or most delicious part of the apple. Yet the core gives the rest of the apple meaning... A coreless apple might be just as attractive to behold and even more luscious to eat, but it would be a biological monstrosity."

Unfortunately the force of this analogy depends upon the assumption that science *is* the core; the analogy may be applied equally well to any other component of a "liberal education" of which the same assumption is made; and at the end of the chapter, again in the language of rhetoric and poetry, what Dr. Glass makes a case for is not science but philosophy:

"What we sadly need today is a philosophy that will embrace the rights and the needs of the individual and also the welfare of society, as the Judaeo-Christian outlook so successfully did for many centuries. At the same time, however, the new must be a dynamic philosophy, changing with the basic shifts in man's scientific understanding of himself and his world, working toward a higher synthesis and not irrevocably tied to Newton or Einstein, Pavlov or Freud, Darwin or Mendel. For all of these however great, have seen through a glass darkly. The whole of nature is far beyond man's present comprehension, the edifice of science and philosophy a mere foundation, and not the completed structure it will some day be. For we hope to build of our ideas and conceptions a cathedral, vast and beautiful, time-tested, wherein the human spirit may find strength and courage, peace and wisdom."

The humanist takes heart.

The third lecture deals with "Darwinian Evolution and Human Values," describes one of the major revolutions in human thought, and again makes very clear the relation of science to the social sciences, to ethics, and to ethical problems and ethical thought. The book ends with its repeated challenge:

"Darwinism, shattering man's belief in himself as a fallen angel, now places in his hands the power to create new forms of life and to remold his own nature. Can we find in ethics the humility that will make that power safe? Man's religious beliefs and his dedication to something higher and greater than himself, must, I hold, become ere long a crucial factor in his further evolution."

It is a humane and thoughtful book, one which may well persuade the humanist of the importance of scientific knowledge and the scientist of the importance of humane learning. Dr. Glass has a great deal of both.

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Radiation, Genes, and Man

By BRUCE WALLACE AND TH. DOBZHANSKY. New York: Henry Holt and Company, 1959, pp. viii + 205, $4.75.

THIS BOOK was written to give the layman the knowledge of radiation and genetics necessary for an understanding, or at least an appreciation, of the problem of assessment of radiation damage in man. For this purpose the authors have succeeded very well.

The first five chapters deal with genes and chromosomes, the rules of heredity, the nature of radiation, and its effect on the genetic material. Chapters 6 and 7 consider population effects and are based primarily on the Haldane-Muller principle. Chapter 8 is entitled "Unsolved Problems", and considers the (remote) possibility of extinction by radiation—induced mutation, and the authors' own controversial notions of the genetic structure of populations. The final chapter gives their conclusions, which are in general agreement with the various committee reports here and abroad.

The authors' view of society's new responsibility in an atomic age is embodied in this quote:

> If society sanctions the use of atomic energy, it must assume the responsibility for the genetic defects resulting therefrom. It must be prepared to care for persons who are afflicted with these defects. We have seen that man-made mutations do not differ in any obvious manner from "natural" mutations. This fact, however, offers no basis for denying the existence of man-made genetic damage. On the contrary, society will have to be prepared to assume responsibility for the care of all persons suffering from severe inherited disorders.
>
> Confronted with this responsibility, society is likely to demand corresponding authority—the authority to prescribe rules of conduct that will tend to keep the incidence of inherited disorders as low as possible. Will people be ready to grant such authority? Involved is some type of regulation of human reproduction and family life. This is a sphere which people have always jealously guarded, and properly so, from any outside authority. Not only compulsion but even persuasion is frequently resented as an unwarranted interference with private life. And yet, it is difficult to see how mankind can ignore this problem much longer.

The approach to population effects in chapters 6 and 7 follows Muller's technique of measuring mutational damage in terms of mutant gene extinctions, or "genetic deaths". Several hypothetical numerical examples are given, and one quote will illustrate how completely this viewpoint is followed: "We must remember that no matter how long it takes to re-establish a genetic equilibrium (after an increased mutation rate), the total damage done by a given number of mutations is determined by that number, and that number alone." The authors make clear elsewhere that this depends on the constancy of the population size and on the assumption that damage is measured in units of gene extinctions. The utility of this assumption is eventually questioned in chapter 8, but only after extensive calculations and discussions based on it in earlier chapters.

For the general reader the book gives a good picture of the problem and of the present state of knowledge. My only serious criticism from this standpoint is that the algebra in chapters 6–8 is unnecessarily complex and sometimes in error, with the result that these chapters are unintelligible to most of the readers for whom the book is intended.

But the book is certain to be read by those with some genetic knowledge and an interest in the details. For this group, it leaves something to be desired. Hence this review, for readers of the *American Journal of Human Genetics*, is mainly critical. This should not be construed as disapproval of the book for the purpose for which it was written.

In several places the calculations are curiously indirect. An example is found on page 131, where the rate of mutation per locus per roentgen in Drosophila is obtained by taking the chromosomal lethal rate and dividing by the number of lethal-producing loci, as estimated mainly from allelism tests. The procedure ignores the single locus Drosophila studies that are directly comparable to Russell's mouse experiments. This indirectness, and perhaps differences between gonial and spermatozoan rates, account for the erroneous implication that the radiation-induced rate is approximately the same in mice and Drosophila. At the bottom of the same page is a troublesome printing slip; "acute" is interchanged with "chronic".

A second example: On page 118, the direct information from human consanguinity studies is ignored in favor of indirect Drosophila calculations of the store of hidden genetic damage in man. A similar oblique approach is found several times in the population genetics section, with the consequence that some problems appear to be more complex than they are.

In several places the writing is loose. A trivial example: On page 16, the title of this excellent illustration would be improved, it seems to me, if *interaction* were replaced by *combined effect*. A more important example is on page 84 where a mutation rate-doubling dose of radiation in Drosophila is compared with seemingly similar values for bacteria, without considering the complications of generation-counting in going from single- to multi-celled organisms, or of the fact that the Drosophila value would have been different if it had been based on gonial radiation.

The most controversial material is in chapter 8. Here the authors contrast the "classical" with the "balance" hypothesis of population structure. The authors state that any attempt to calculate the effect of mutation on fitness under the "classical" hypothesis implies the assumption of an "Ideal Man"—a Superman, or a Platonic ideal, completely homozygous at all loci and perfectly fitted to his environment. Why? Isn't it quite proper to compare in principle the fitness of a genotype, or the average of a population of genotypes, with what it would be if deleterious mutants or homozygous segregants from selectively maintained polymorphic systems were absent? This doesn't imply homozygosity at all loci, or in fact anything at all about loci not contributing to the effect being measured.

Wallace and Dobzhansky then put forth as an alternative their "balance hypothesis", familiar to geneticists from their recent technical writings. They assume that the typical locus consists of a large number of mutually heterotic alleles, and that "every gene is potentially heterotic and potentially deleterious". This seems highly dubious, for there must be a large number of mutants that are harmful in any environment they will ever encounter. To me it seems probable that the great majority of mutations are to alleles that are not part of and don't fit into a selectively maintained polymorphic system. This is certainly true of mutations whose effects are sufficiently large to be studied individually, and is to be expected on physiological grounds. This is not to argue that loci where heterotic alleles are maintained are non-existent or unimportant in populations; quite the contrary, for they exert an effect on the population variance out of all proportion to their frequency of initial occurrence.

The authors note that, with a hypothetical system of mutually heterotic alleles, the larger the number of alleles maintained, the greater the average population fitness. They make the interesting suggestion that mutation may enhance the immediate fitness by replacing alleles that are lost through random drift. The problem of random drift does become increasingly important with a very large number of alleles for, in the absence of selection, the rate of decay of a system of n alleles when a steady rate is reached is $n(n-1)/4N$ where N is the effective population number. The theoretical question of the effect of mutation on fitness in a system of n mutually heterotic alleles in a finite population is a difficult one.

However, it seems clear that on most polyallelic models, where gene frequencies are determined mainly by selective balance, mutation will reduce fitness, since it will generally carry the frequencies away from their equilibrium values at which fitness is assumed to be maximized. Furthermore, the reduction in fitness is proportional to the mutation rate. Hence the usual effect of mutation, except in contrived situations (such as equal fitness of all homozygotes and equal mutation rates for all alleles as Wallace

and Dobzhansky assume), will be to reduce the fitness. The role of mutation in a heterotic system is qualitatively the same as for a "classical" system. It is the source of genetic variability, but its immediate effect is to lower the fitness. To assume otherwise is to remove the creative role in evolution from selection and assign it to mutation.

Wallace and Dobzhansky say that probably the greatest difficulty in assessing the human risk from radiation is uncertainty as to how many loci are "classical" and how many heterotic. I agree that this is an interesting and important question. But this does not seem to me to be *the* major difficulty in measuring the human risk. If, for example, we make the extreme assumption that as many as 50 per cent of the measured induced mutations in a natural population are to alleles that are part of a selectively maintained polymorphic system, the calculations on the "classical" view would be off by less than 50 per cent. Considering all the other uncertainties, this isn't very large; I think most geneticists would be delighted with this much accuracy. There is a large area of uncertainty about genes with small effects—isoalleles and polygenes; their contribution to total fitness and human welfare, their mutation rates, how they interact with each other and with the environment, and how they are selected in characters, such as size, where the optimum is intermediate. The problem seems to me to be much more complex than the question Wallace and Dobzhansky ask.

In my view, still greater uncertainties arise from difficulties in getting direct measurements of human mutational effect, from lack of knowledge of the distribution of such effects in time, and from ignorance of the conversion factors to relate gene extinctions to specific and tangible measures of human suffering and frustration.

Appended here are a few mathematical points that may be of interest to some readers:

p 123. Not 20 generations, but about 14.

p 136. The error lies not in the omission of $(nu')^2$, but in ignoring selection during the first n generations in the derivation 2 pages earlier. Here, and elsewhere, the approximate expressions seem to me to be harder to understand than the exact ones, which would also eliminate such errors as this.

p 147. The formula on line 5 should be only half as large, if my understanding is correct.

p 165. I think the formulae $x'u$ and $2x'u$ should be $D_i u'$ and $2D_i u'$, and if so, the concluding sentence in this paragraph is wrong.

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Hemophilia and Other Hemorrhagic States

Edited by KENNETH M. BRINKHOUSE. University of North Carolina Press, 1959, 266 pp., $7.50.

Hemophilia and other Hemorrhagic States is the record of the symposium on hemophilia held in Rome in September, 1958. The 27 papers are an accurate reflection of the currents of thinking about hemorrhagic disease at that time. The casual reader will be struck by the apparent deceleration of the generation of new ideas compared to the 1940's, and by the decrease in acrimonious discussion which marred the literature of that period.

Several of the studies are of particular interest to the geneticist. Pavlovsky describes two young girls, daughters of hemophiliacs, who have clinical and laboratory evidences

of classic hemophilia. These girls resemble McGovern and Steinberg's symptomatic hemophilic carrier rather than the true homozygous female hemophiliacs described by Merskey and others.

Considerable attention is given to newer views concerning von Willebrand's disease, a hemorrhagic disorder in which symptoms of variable severity occur in both males and females. This syndrome, transmitted by an abnormal dominant autosomal gene, is one of the several in which the bleeding time is greatly prolonged. Nilsson and Blombäch describe their studies in von Willebrand's original cases in the Åland Islands, demonstrating a partial deficiency of antihemophilic factor. Their experiments raise many questions, for a fraction of normal plasma devoid of antihemophilic activity is said to correct the bleeding defect in these patients. Re-examination of some of the cases previously classified as instances of pseudohemophilia—that is, of a disorder limited to the blood vessels—has demonstrated the presence of coagulative defects similar to those in the Åland Islanders. Several of the authors attempt to unravel the confusion resulting from the subdivision of these cases.

Graham and his associates report the first example of an "acquired" isolated deficiency of Stuart factor. Previously only congenital cases had been reported, although a deficiency of Stuart factor in combination with other deficiencies of the Vitamin K-dependent clotting factors has been observed in a number of different clinical situations. McCain, Chernoff and Graham also confirmed the previously reported mode of inheritance of Hageman trait. This coagulative disorder, a deficiency of Hageman factor, seems to be due to the inheritance of autosomal recessive genes. As has been true of our own experience, only a few presumable heterozygotes can be identified in the laboratory.

The remainder of the volume deals with clinical and experimental aspects of the hemophilia-like diseases. The volume is highly readable and well illustrated, but unfortunately the discussions of the various papers have been omitted. This is a little like leaving the icing off the cake. The food for thought has been published, but what makes its ingestion enjoyable has been omitted.

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Outline of Human Genetics

By L. S. PENROSE. New York: John Wiley and Sons, 1959, xii + 146 pp., $2.50.

PENROSE has written a short, clear description of human genetics for nongeneticists. The topics covered include the basic principles of the inheritance of various characteristics in man and the side issues of the application of these principles to anthropology and eugenics.

The virtue of this book is in its brevity. Intelligent persons who wish to acquire a general knowledge of human genetics will certainly find it here. In particular, this should be an excellent vehicle for introducing human genetics to persons who require genetic counselling. However, because very few conditions are treated at length, it will be necessary to supplement reading in this book with a specific discussion concerning the problem at hand.

The last of the six chapters, Eugenics and Dysgenics, presents a nebulous treatment of many of the fascinating problems that are found in this field. The correlation in recent years between low intellect and high fertility is generalized to "all other civilizations".

Penrose fails to observe that fertility (number of offspring) is not a measure of fecundity (ability to have offspring). This gets him into the ludicrous position of praising persons "lacking in the qualities considered socially desirable," because these are the fertile persons whose progeny will make up much of the next generation. On these premises, his hope for positive eugenics seems limited to waiting for the day when the transduction or directed mutation of desirable genes become realities.

Negative eugenics has fared no better than positive eugenics. There is no notice of the fact that this is a highly effective measure against a few rare dominant traits, and for traits for which there is a definite genetic component of uncertain magnitude, such as feeblemindedness, there is a good deal of error in stating that "to be effective, (negative eugenics) must catch the individuals it wishes to discourage before they start breeding."

In general, the level of writing is excellent. Subsequent editions might be improved by the omission of the table on page 18, which introduces crossing over long before the text is ready to take up the subject, and the appendices, which are comprehensible largely to persons who already understand the subject of population genetics.

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Essential Tremor: A Clinical and Genetic Population Study

By Tage Larsson and Torsten Sjögren. Copenhagen: E. Munksgaard, 1960, pp. 176, 42 Tables, 6 Figures.

In another fine contribution to the demographic inventory of human genetics, the Scandinavian investigators used the North Swedish population which had previously been studied with regard to various forms of mental illness and mental defect. The relatively large number of essential tremor cases observed (191 families with 210 affected children and 177 affected parents), a low migration rate (most families had been local residents for several centuries), and carefully annotated parish records were important prerequisites for this thorough genetic analysis.

Regarded as a well-defined disease entity, essential (familial) tremor was found to manifest itself around the age of 50. The tremor usually began in the hands and tended to be slowly progressive, spreading to the arms, face and tongue and finally to the trunk and legs. In line with the observations of previous investigators, tremor was the only symptom in the majority of cases. In the present study, 21 per cent of the patients revealed additional neurological symptoms, although there was no instance of other neurological disease with a tremor pattern in the relatives of affected persons. Therefore, the occurrence of other neurological disturbances (rigidity, stiff gait, etc.) was ascribed to aging and the progression of the original disease, although a coincidental association with another process could not be excluded. The sample of essential tremor cases ascertained from a relatively isolated parish population by means of clinical examination or intensive interviews with relatives consisted of a total of 210 patients, 105 of whom were still alive at the time of the study. Personal examination was possible in 81 cases. Geneaological data were obtained for all registered cases in order to determine whether their relatives had been affected or unaffected.

In this manner the disease was traced to nine ancestral couples, most of whom were born before 1800. If the mode of inheritance involved followed strictly the pattern of autosomal dominance, four ancestral pairs were responsible for all but two affected per-

sons. While the frequency of the trait in the western sector of the parish was estimated at 8–9 per cent, affected persons revealed no evidence of excess mortality or reduced fertility.

Females were less frequently affected than males (84: 126) and showed a moderately higher average age of onset, presumably because of their more protected station in life. While the authors emphasize the fact that males are required to perform more difficult manual tasks than females, it should be borne in mind that the observed difference between the sexes may have been biological rather than social in origin. According to life span data in man and lower animals, females demonstrate a propensity for greater biological vigor, possibly due to an advantage conferred by the second X chromosome.

Of particular interest was the observation that the essential tremor pattern in this isolated population differed clinically from that seen in another group of families in an adjacent parish. The authors hypothesize that essential tremor cases in different geographical areas may vary in symptomatology because they arise from different genotypes. However, confirmation of this hypothesis will have to await further refinement of diagnostic procedures.

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LETTERS TO THE EDITOR

Chronic Hereditary Nephritis and Y-Chromosome linkage: Reply to Graham

May 4, 1960

To the Editor
Dear Sir:

In the December issue of the *American Journal of Human Genetics*, Graham (1959) discussed our kindred of chronic hereditary nephritis (Perkoff et al. 1958). Whereas we had considered the data to be compatible with a partially sex-linked dominant trait, he concluded the an autosomal dominant gene with incomplete penetrance and a lethal effect in involved male children was a more likely explanation.

Several points raised by Graham call for comment. First, we do not believe that the genetic anomaly for hereditary nephritis leads to the appearance of two renal disorders in a single family. As we have discussed in detail (Perkoff et al. 1958), we consider this disease to be separate from either classic pyelonephritis or glomerulonephritis. In addition to evidence already cited to favor this view, another family with hereditary nephritis has been reported to show a varied renal histopathology with fat-filled cells similar to those noted in our cases (Goldman and Haberfelde, 1959). Second, Graham has assumed that all the members of the pedigree who have nerve deafness without renal disease carry the same gene as those with renal disease. While we agree that this may be true, the evidence on this point is not sufficient to allow a definite decision at the present time. Also, in making this assumption, Graham has included in his calculations individuals we specifically excluded for various reasons. As an example, may we cite III-18 and III-23. Their father, who was not a member of the pedigree, had otosclerosis. Graham considered the children of these subjects as families of involved males. However, we considered them as uninvolved since neither III-18 nor III-23 nor their children have any evidence of renal disease or of nerve deafness.

The most important issue, however, relates to the mechanism of inheritance itself. The essential data are shown in the following table.

OFFSPRING OF AFFECTED MALES AND FEMALES IN THE KINDRED
(Including Known Carriers)

	From 26 Affected Females		From 12 Affected Males	
	Affected	Normal	Affected	Normal
Female	41	43	24	5
Male	24	37	2	18

From these data it is apparent, on the basis of a simple dominant trait, that there is a deficiency of affected male offspring from involved females, a finding which could be due to a lethal gene or to chance. In the offspring of affected males, however, not only are most of the males normal, but most of the females are affected. One could say, in this instance, there is a deficiency of *normal* females, as well as of affected males, but it would be difficult to ascribe this finding to the effects of a lethal gene. Finally, although the possibility of early fetal death exists, there is no history of frequent abortions, stillbirths or deaths in childhood to suggest the presence of a lethal gene.

We recognize that there is controversy concerning the possibility of crossing over between the X and Y chromosomes in the human (Sachs, 1954, Ford and Hamerton, 1956). However, the data in our kindred seem to fit best with the findings expected of a sex-linked dominant trait with a few instances of crossing over. Perhaps future study of the deaf individuals and those thought to represent instances of crossing over may yield information of a *positive*

character which can be used to construct a satisfactory alternative hypothesis of the inheritance in this kindred. At the present time we do not believe this can be done.

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Chronic Hereditary Nephritis: Not Shown to be Partially Sex-Linked

May 20, 1960

To the Editor
Dear Sir:

After reviewing the papers of Perkoff et al. and my discussion of them, I realize that I may have misled some of your readers. Many of my generalizations about "hereditary chronic kidney disease" were mere restatements of their findings. My remarks were intended to orient readers unfamiliar with this interesting disorder, but I can see that the unwary reader might have concluded erroneously that I, not the Utah workers, was the expert on renal disease. The critical reader can only admire their work. It was superbly executed and has been very illuminating. We are in disagreement because I believe their work has been more illuminating than they will admit. I want to join them in affirming that this type of nephropathy is probably unique and cannot be described adequately with the categories (glomerulonephritis, pyelonephritis, nephrosclerosis) conventionally used by students of renal disease.

In examining their latest paper (Perkoff et al., 1958) I observed a statistically significant reversal of the sex ratio (Graham, 1959). This had been overlooked by the authors originally and, incidentally, has been ignored in the letter above. The correct genetic hypothesis, in my opinion, must account for this fact. The Utah workers have not yet shown us how the hypothesis of partial sex-linkage accomplishes this.

Table 1 in their letter above differs somewhat from Table 4 in my publication, because they considered the distribution of *all* children of affected persons while I considered *only* the children in Generation IV, the largest single generation. This restriction was chosen not to favor my hypothesis, but because persons in the same generation are more apt to be affected similarly by the environment, and observation of living persons is more reliable than hearsay evidence. The restriction itself did not produce the reversed sex ratio, however, as will be seen below. Here the authors' own table has been rearranged and the discrepancy is still apparent.

OFFSPRING OF AFFECTED MALES AND FEMALES IN THE KINDRED

Affected Parents	Sons			Daughters			Total
	Normal	Affected	Total	Normal	Affected	Total	
26 Affected Mothers	37	24(26)	61(63)	43	41	84	145(147)
12 (14) Affected Fathers	18(20)	2(3)	21(23)	5(10)	24	29(34)	49(57)
Totals	55(57)	26(29)	81(86)	48(53)	65	113(118)	194(204)

Figures in parentheses result from the decision to score III-18 and III-23 as affected and include their progeny.

The ratio of sons to daughters of affected persons in the bottom line of the rearranged table is 81:113. The χ^2 value (unity expected) is 5.3, chance probability about 3%. Also the ratio of normal:affected sons (55:26) is significantly different from unity, while that among daughters (48:65) is not. The top line shows that there is a *deficiency of affected sons from affected mothers*, and the middle line shows that *there is a deficiency of affected sons from affected fathers* also. The observation from which the authors postulate partial sex-linkage is seen in the middle line. Here the *deficiency of affected sons* from affected fathers is accompanied by a large *excess of affected daughters*.

If I understand them correctly, the Utah group are saying in effect that the ratio 5:24 (5 normal daughters:24 affected daughters among the daughters of affected fathers) is crucially significant while the ratio 81:113 (sons:daughters among children of affected parents) can be ignored as a chance event. I, on the other hand, maintain that both abnormal ratios must be accounted for. I assign greater importance to reversal of the sex ratio and tend to depreciate the significance of the 5:24 ratio for two reasons: 1) The population producing the sex ratio reversal is large and that producing the other ratio is small. 2) The 5:24 ratio would be 10:24 (parenthetical additions to the rearranged table) if the deaf males III-18 and III-23 are scored as transmitters and their children included. These men must be examined carefully before a decision is made because it is much easier to consider a 10:24 ratio the result of chance than a 5:24 ratio. Their genotype is questionable for three reasons: a) The mother of these men transmitted kidney disease because they have affected siblings; b) Their first cousin (III-7) was only deaf and produced children with renal disease; c) One parent in 16 transmitting an autosomal dominant is expected to have 4 normal children, as each of these men does.

These two men have been scored as normal at this locus by the authors on the grounds that the deafness is different clinically from that of the other deaf persons, and their father had otosclerosis. Thus the 5:24 ratio hangs by a slender thread. All things considered, I am loath to accept a cytologically questionable genetic hypothesis when it does not explain all the findings (sex ratio) and when at the same time considerable fault can be picked with the crucial evidence. (I admit to uneasiness in coming to this conclusion but am willing to risk letting future kindreds bear out my prescience.)

My suggestion that excessive numbers of affected males may have died early *in utero* is apparently erroneous, but this does not affect the validity of the preceding argument. One possible explanation of the sex ratio reversal is discarded but this does not reverse the ratio! The deficiency of males still exists and must be accounted for. There are other possible mechanisms as was pointed out at the 1960 meeting of the American Society of Human Genetics by Dr. Richard Shaw of the University of Virginia. He was able to conceive of at least two others.

I suggest that the evidence which will settle the problem of the inheritance of this pathologic state is not yet in, but the situation is hopeful. If study and reporting of additional kindreds can be stimulated, it is possible that the crucial genetic information will be en-

countered by someone well before the time when it might be furnished by the hypothetical cross-overs in Utah.

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A Proposed Standard System of Nomenclature of Human Mitotic Chromosomes

To the Editor.

Dear Sir:

The rapid growth of knowledge of human chromosomes in several laboratories, following advances in technical methods, has given rise to several systems by which the chromosomes are named. This has led to confusion in the literature and so to the need for resolving the differences. Consequently, at the suggestion of Dr. C. E. Ford, a small study group was convened to attempt the formulation of a common system of nomenclature. The meeting was arranged, through the good offices of Dr. T. T. Puck, to be held at Denver, in the University of Colorado, under the auspices of the Medical School. The meeting of this study group was made possible by the support of the American Cancer Society, to whom grateful thanks are due. For practical reasons, it was decided to keep the group as small as possible and to limit it to those human cytologists who had already published karyotypes.* In addition, three counselors were invited to join the group to guide and aid the discussions and, if necessary, to arbitrate. Fortunately, the last office did not prove necessary, and it was possible by mutual agreement to arrive at a common system which has flexibility.

It was agreed that the principles to be observed by the system should be simplicity and freedom, as far as possible, from ambiguity and risks of confusion, especially with other systems of nomenclature in human genetics. It should also be capable of adjustment and expansion to meet the needs of new knowledge of human chromosomes. The system should be agreed to by the greatest possible proportion of cytologists working in the field, but the risk that a minority may be unable to accept the system as a whole should not be allowed to delay adoption by a majority.

It was agreed that the autosomes should be serially numbered, 1 to 22, as nearly as possible in descending order of length, consistent with operational conveniences of identification by other criteria. The sex chromosomes should continue to be referred to as X and Y, rather than by a number, which would be an additional and ultimately, a superfluous appellation.

It was generally agreed that the 22 autosomes can be classified into seven groups, distinction between which can readily be made. Within these groups, further identification of individual chromosomes can in many cases be made relatively easily. Within some groups,

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* In contemporary publications the terms, karyotype and idiogram, have often been used indiscriminately. We would recommend that the term, *karyotype*, should be applied to a systematized array of the chromosomes of a single cell prepared either by drawing or by photography, with the extension in meaning that the chromosomes of a single cell can typify the chromosomes of an individual or even a species. The term, *idiogram*, would then be reserved for the diagrammatic representation of a karyotype, which may be based on measurements of the chromosomes in several or many cells.

TABLE 1. CONSPECTUS OF HUMAN MITOTIC CHROMOSOMES

Group 1–3	Large chromosomes with approximately median centromeres. The three chromosomes are readily distinguished from each other by size and centromere position.
Group 4–5	Large chromosomes with submedian centromeres. The two chromosomes are difficult to distinguish, but chromosome 4 is slightly longer.
Group 6–12	Medium sized chromosomes with submedian centromeres. The X chromosome resembles the longer chromosomes in this group, especially chromosome 6, from which it is difficult to distinguish. This large group is the one which presents major difficulty in identification of individual chromosomes.
Group 13–15	Medium sized chromosomes with nearly terminal centromeres ("acrocentric" chromosomes). Chromosome 13 has a prominent satellite on the short arm. Chromosome 14 has a small satellite on the short arm. No satellite has been detected on chromosome 15.
Group 16–18	Rather short chromosomes with approximately median (in chromosome 16) or submedian centromeres.
Group 19–20	Short chromosomes with approximately median centromeres.
Group 21–22	Very short, acrocentric chromosomes. Chromosome 21 has a satellite on its short arm. The Y chromosome is similar to these chromosomes.

especially the group of chromosomes numbered 6–12, including also the X chromosome, the distinctions between the chromosomes are very difficult to make by presently available criteria. However, lesser difficulties are encountered in separating chromosomes 6 and the X from the remainder of this group. It is believed that, with very favorable preparations, distinction can be made between most, if not all, chromosomes.

It is proposed that the autosomes first be ordered by placing the seven groups as nearly as possible in descending order of size. Within each group the chromosomes are arranged, for the most part, by size. It was desired specifically to avoid the implication that size relationships have been permanently decided in every instance, but it is hoped that the assignment of numbers will be permanently fixed. In those cases where distinction is at present doubtful, final definition of each chromosome can be left until further knowledge has accrued, though an attempt is made to provide a characterization of each. These principles make it possible to draw up a conspectus of the chromosomes, a table of their quantitative characteristics and a table of the synonyms which authors have already published. These are appended (Tables 1, 2 and 3).

In Table 2, showing the diagnostic characters of the chromosomes, three parameters are relied upon. These are: 1) The length of each chromosome relative to the total length of a normal, X-containing, haploid set, i.e., the sum of the lengths of the 22 autosomes and of the X chromosome, expressed per thousand; 2) The arm ratio of the chromosomes expressed as the length of the longer arm relative to the shorter one; and 3) The centromeric index expressed as the ratio of the length of the shorter arm to the whole length of the chromosome. The two latter indices are, of course, related algebraically quite simply, but it is thought useful to present both here. In some chromosomes, the additional criterion of the presence of a satellite is available (Table 1), but in view of the apparent morphological variation of satellites, they and their connecting strands are excluded in computing the indices.

Table 2 shows the range of measurements determined by various workers. Some of the variation expresses the uncertainty due to measurement of relatively small objects; but many of the discrepancies between different workers' observations are due to the measurement of chromosomes at different stages of mitosis and to the effect of different methods of pretreatment and preparation for microscopic study. The ranges shown, therefore, represent the maxima and minima of the means found by different workers using different techniques. However, within any one worker's observations, the variations are not so broad.

TABLE 2. QUANTITATIVE CHARACTERISTICS OF THE HUMAN MITOTIC CHROMOSOMES

All measurements were made from cells of normal individuals, except those made by Fraccaro and Lindsten, which included cases of Turner's Syndrome. The column A is the relative length of each chromosome, B is the arm ratio and C the centromere index, as defined in the text.

	Tjio and Puck (6)			Chu and Giles (2)			Levan and Hsu (5)			Fraccaro and Lindsten*			Lejeune and Turpin (4)*			Buckton, Jacobs, and Harnden*			Range		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1	90	1.1	48	90	1.1	48	85	1.1	49	82	1.1	48	87	1.1	48	83	1.1	48	82–90	1.1	48–49
2	82	1.6	39	83	1.5	40	79	1.6	38	77	1.5	40	84	1.5	40	79	1.6	38	77–84	1.5–1.6	38–40
3	70	1.2	45	72	1.2	46	69	1.2	45	65	1.2	45	67	1.2	46	63	1.2	46	63–72	1.2	45–46
4	64	2.9	26	63	2.9	26	63	2.7	27	62	2.6	28	62	2.6	25	60	2.6	28	60–64	2.6–2.9	25–28
5	58	3.2	24	58	3.2	24	59	2.6	28	60	2.4	29	57	2.4	30	57	2.4	30	57–60	2.4–3.2	24–30
X	59	1.9	34	57	2.8	38	52	1.6	38	54	1.6	38	58	2.2	32	51	1.7	37	51–59	1.6–2.8	32–38
6	55	1.7	37	56	1.8	36	56	1.7	37	54	1.6	38	56	1.7	37	56	1.6	38	54–56	1.6–1.8	36–38
7	47	1.3	43	52	1.9	35	51	1.9	35	50	1.7	37	51	1.8	36	50	1.7	37	47–52	1.3–1.9	35–43
8	44	1.5	29	46	1.7	29	48	1.6	33	47	1.7	37	48	2.4	29	46	1.5	40	44–48	1.5–2.4	29–40
9	44	1.9	40	46	2.4	38	47	1.8	36	45	2.0	33	47	1.9	35	44	2.1	32	44–47	1.8–2.4	32–40
10	43	2.4	27	45	2.3	30	45	2.0	33	45	2.6	34	45	2.6	27	44	1.9	35	43–45	1.9–2.6	27–35
11	43	2.8	34	44	2.1	32	44	2.2	31	43	2.2	31	44	1.6	39	43	1.5	40	43–44	1.5–2.8	31–40
12	42	3.1	24	43	3.1	24	42	1.7	32	43	1.7	37	42	2.8	27	42	2.1	32	42–43	1.7–3.1	24–37
13	35	8.0	11	32	9.7	10	32	5.0	16	34	4.8	17	33	6.8	14	36	4.9	17	32–36	4.8–9.7	10–17
14	32	7.3	12	34	9.5	9	37	4.0	18	35	4.4	19	32	7.0	13	34	4.3	19	32–37	4.3–9.5	9–19
15	29	10.5	9	31	11.9	8	35	4.7	17	33	4.6	22	31	10.0	9	34	3.8	22	29–35	3.8–11.9	8–22
16	32	1.8	36	27	1.6	38	30	1.4	42	31	1.4	42	29	1.4	41	33	1.4	31	27–33	1.4–1.8	31–42
17	29	2.8	26	30	2.1	33	29	2.4	30	30	1.9	35	29	3.1	23	30	1.8	36	29–30	1.8–3.1	23–36
18	24	3.8	21	25	3.8	22	25	2.6	28	27	2.5	29	26	4.2	21	27	2.4	29	24–27	2.4–4.2	21–29
19	22	1.4	41	22	1.9	34	24	1.2	40	25	1.3	43	22	1.4	42	26	1.2	45	22–26	1.2–1.9	34–45
20	21	1.3	44	19	1.3	44	21	1.2	40	23	1.3	43	20	1.2	43	25	1.2	46	19–25	1.2–1.3	40–46
21	18	3.7	21	15	6.8	13	13	2.5	28	19	2.5	29	15	2.3	31	20	2.5	29	13–20	2.3–6.8	13–31
22	17	3.3	23	12	6.0	14	16	2.0	33	17	2.3	30	13	4.0	20	18	2.7	27	12–18	2.0–6.0	14–33
Y	19	∞	0	11	∞	0	18	4.9	17	22	2.9	26	18	∞	0	18	4.9	17	11–22	2.9–∞	0–26

* Unpublished data.

Reference should be made to two other matters of nomenclature. In the first place, it is considered that no separate nomenclature for the groups is needed. It is considered that any group to which it may be necessary to refer will be a sequence of those designated by Arabic numerals. Hence, any chromosome group may be referred to by the Arabic numerals of the extreme chromosomes of the group, joined together by a hyphen, e.g., the group of the three longest chromosomes would be Group 1–3. This scheme has the merit of great flexibility. For instance, chromosomes X and 6 may be separated from the Group 6–12 whenever they can be distinguished.

Secondly, there is the problem raised by the abnormal chromosomes which are being

TABLE 3. SYNONYMY OF CHROMOSOMES AS PUBLISHED BY VARIOUS WORKERS

New Chromosome Number	Tjio and Puck (6)	Chu and Giles (2)	Levan and Hsu (5)	Ford, Jacobs and Lajtha (3)	Böök, Fraccaro and Lindsten (1)	Lejeune, Turpin and Gautier (4)
1	1	1	1	1	1	G1
2	2	2	2	2	2	G2
3	3	3	3	3	3	G3
4	4	4	4	4	4	G4
5	5	5	5	5	5	G5
6	6	6	6	6*	6	M1
7	7	7	7	(8)	7	M2
8	8	8	8	(9)	8	Md1
9	9	9	9	(11)	9	M3
10	10	10	10	10	10	Md2
11	11	11	11	(12)	11	M4
12	12	12	12	(13)	12	Md3
13	18	14	20	14	14	T1
14	19	15	18	15	15	T2
15	20	13	19	16	13	T3
16	13	17	15	19	16	C1
17	14	16	13	17	17	P1
18	15	18	14	18	18	P2
19	16	19	16	20	19	C2
20	17	20	17	21	20	C3
21	21	21	22	22	21	Vh
22	22	22	21	23	22	Vs
X	X	X	X	?(7)	X	X
Y	Y	Y	Y	Y	Y	Y

* In the published idiogram the chromosomes of group 6–12 (including X) were indicated by discontinuous lines and left unnumbered owing to the uncertainty of discrimination at that time. For the purpose of this table, these chromosomes have been assigned the numbers shown in brackets, in serial order of length.

encountered in the more recent studies. Their nomenclature was discussed without a definite conclusion being reached. Broadly, it was agreed, however, that any symbol used should avoid incorporating a specific interpretation which was not reasonably established. It was suggested that arbitrary symbols, prefixed by a designation of the laboratory of origin, should usually be assigned to the abnormal chromosome.

In this connection, two further requisites for coordination of research were discussed. One is the storage of documentation for reference, perhaps in a central depository, additional to what it may be possible to publish. The other is the desirability that cultures be preserved, by the satisfactory methods now used, so that they are available for reference, comparison and exchange.

Some consideration was also given to the desirability of using a uniform system for presenting karyotypes and idiograms, but recognizing that individual variation in taste is in-

volved, rigidity of design was thought undesirable. However, it was recommended that the chromosomes should be arranged in numerical order, with the sex chromosomes near to but separated from the autosomes they resemble. It is desirable that similar ones be grouped together with their centromeres aligned.

It is recognized that choice between the different possible schemes of nomenclature is arbitrary, but that uniformity for ease of reference is essential. Hence, individual preferences have been subordinated to the common good in reaching this agreement. This human chromosome study group therefore agrees to use this notation and recommends that any who prefer to use any other scheme should, at the same time, also refer to the standard system proposed here.

We are well aware of the wide interest in the work of this study group and realize that this meeting is merely a preliminary to a larger meeting. It is believed that two needs have to be met in this respect. One is for seminars and workshops at which workers in the field may exchange information; such seminars are best arranged regionally. The second need, which may come later, is for international conferences; and we believe that Congresses and other organizations whose interests include human genetics, should promote such meetings.

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